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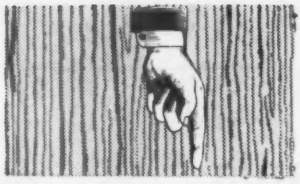

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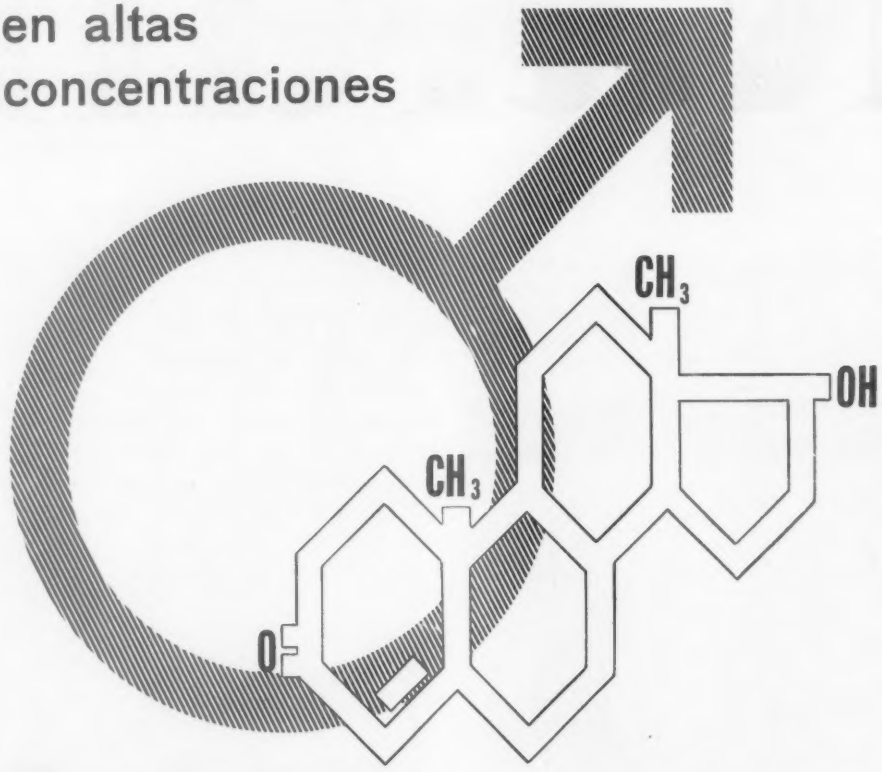

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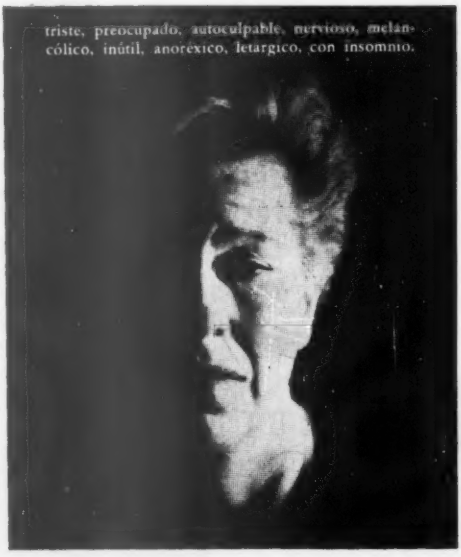

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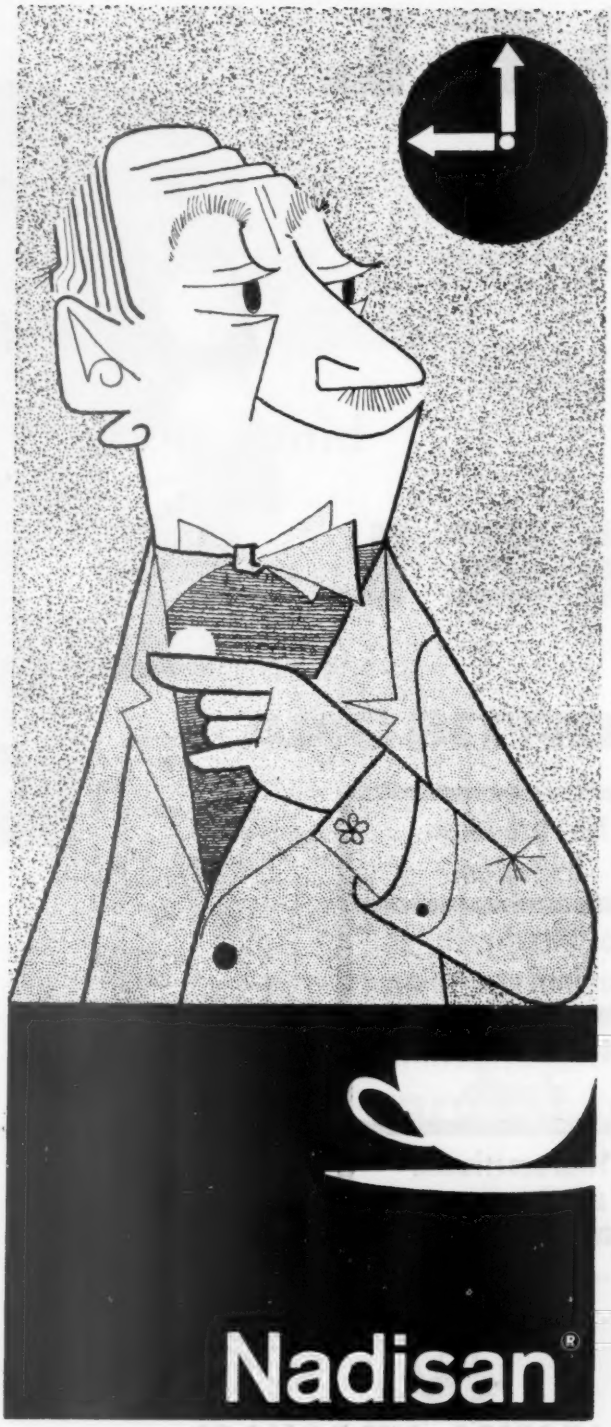

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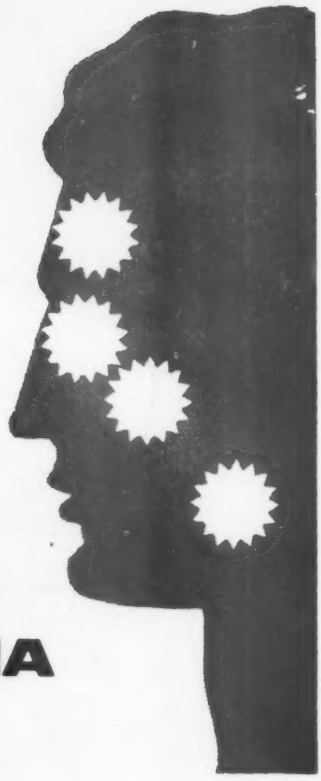

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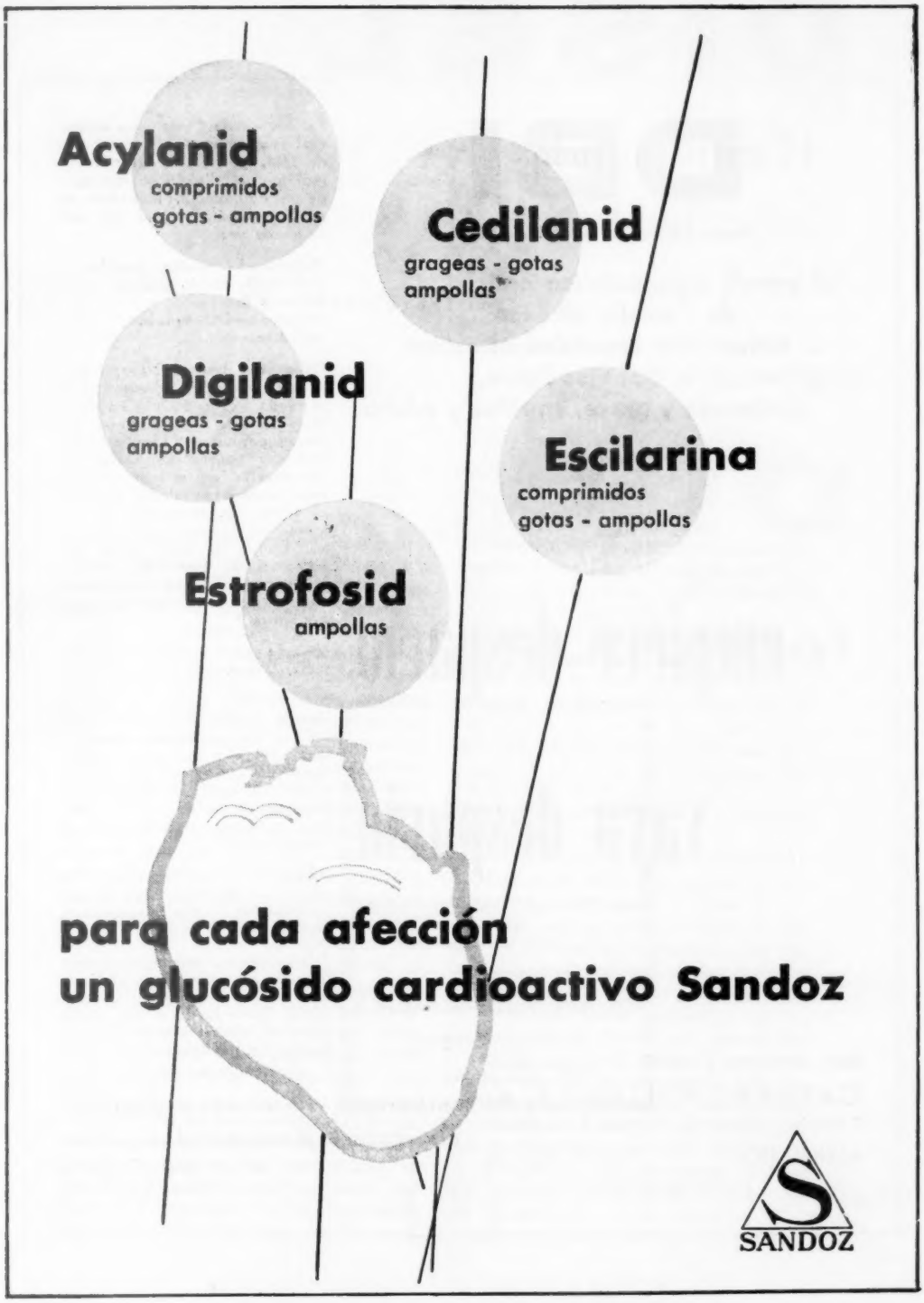
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The introductory paragraphs should state the purpose of the investigation and its relation to other work in the same field, but extensive reviews of the literature should not be given. A brief statement of the principal findings is helpful to the reader.

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Every paper must conclude with a brief summary in which the essential results of the investigation are succintly outlined.

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(1) Notes on preparation of illustrations. *Biochem. J.*, 1956, *62*.
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THE FUNCTIONAL DISCONTINUITIES OF THE AURICULOVENTRICULAR NODE (*)

JESÚS ALANÍS, JUAN J. MANDOKI and GUILLERMO PILAR (**)
(Department of Physiology of the Instituto Nacional de Cardiología, México, D.F.)

IN a previous publication (Alanís González and López, 1959) evidence was presented suggesting the existence of two functional discontinuities in the auriculo-ventricular node (A. V. node): one located between the auricle and the A. V. node and the other between the A. V. node and the bundle of His. The second discontinuity was found to be the most vulnerable in the propagation of impulses from the atrium to the ventricle.

The present study deals with the effects of some factors on these discontinuities.

METHOD

The experiments were performed on isolated and perfused hearts of dogs, according to the technique previously described (Alanís, González and López, 1958; Alanís, López, Mandoki and Pilar, 1959). The electrical activity of the region where the A. V. node and the bundle of His are located, was recorded by means of a pair of steel needle-electrodes, 4-5 mm long, with their tips sharpened to a diameter of 4-12 μ. The needles were covered with an isolating material (Polystyrene) leaving only the tips uncovered. The potentials obtained were amplified by an a. c. —coupled push-pull preamplifier which led to a double-beam cathode-ray oscilloscope. The temperature of the perfusing blood was kept constant, usually at 32° C, in order to have a slow spontaneous activity of the heart, and to be able to explore more extensively the recovery process of the propagation. In some instances, the zone of the A. V. node was cooled by means of a fine jet of cold saline solution (NaCl, 0.7 g per 100 ml). This solution had a temperature of 3-5° C and was applied continuously during several seconds.

The right auricle was stimulated by rectangular pulses, passed through an isolating unit in order to eliminate the connection to ground. In the experiments in which pairs of stimuli were applied, the second stimulus was usual-

(*) This work was supported by a grant from the Life Insurance Medical Research Fund.

(**) Fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

Received for publication, April 1st., 1960.

ly 3 to 5 times the threshold. Adrenaline was injected directly into the aortic tube of the isolated heart.

Results

The functional refractory period of different cardiac structures. — Pairs of stimuli (S_1-S_2) were applied to the auricle at a constant frequency (1 to 1.5 per second). The corresponding impulses were propagated from the auricle to the A. V. node, the bundle of His and the ventricle. The interval between S_1 and S_2 was varied and plotted against the interval between the corresponding electrograms of the auricle (A_1-A_2), of the A. V. node (N_1-N_2); of the bundle of His (H_1-H_2) and of the ventricle (V_1-V_2).

This experimental procedure made it possible to measure the functional refractory period (F. R. P.) for the propagation of impulses through these structures. The F. R. P. has been defined as the minimal interval between two propagated responses (Rosenblueth, Alanís and Mandoki, 1949). The criterion used for measuring these refractory periods was the one proposed previously (Rosenblueth, Alanís and Mandoki, 1949; Rosenblueth, 1958) for both homogeneous and heterogeneous systems. It consists in measuring the longest interval between stimuli which produce the shortest interval between the corresponding responses. This longest interval between stimuli would correspond to the F. R. P. (abscissa of the junction of the right descending branch with the horizontal part of the curve in fig. 1).

At the temperature and the frequency of activation mentioned above, the mean values of the F. R. P's were the following: for the auricle, 180 msec; for the propagation from auricle to A-V node, 320 msec; for the propagation from A-V node bundle of His, 440 msec. The F. R. P. for the propagation from auricle to ventricle was always the same as that from the A. V. node to the bundle of His.

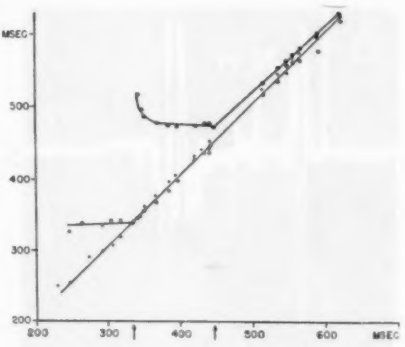

FIG. 1. — *The functional refractory period for the propagation from auricle to the A. V. node and from this node to the bundle of His. Pairs of stimuli (S_1, S_2) applied to the auricle with variable delay. Abscissae, interval between S_1 and S_2 in msec. Ordinates, intervals between responses (in msec.) of the auricle (crosses), A. V. node (circles) and bundle of His (dots). The frequency of S_1 was 1.2/sec. The arrows under the abscissae indicate the values of the F. R. P. (the abscissa corresponding to the junction between the descending and the horizontal branch of the curves). Note the ascending branch (left branch) in the H_1-H_2 curve.*

Factors which modified the propagation of impulses through the A.V. node. — The A. V. node region of a heart driven at a constant frequency was cooled by means of a jet of saline solution until evident changes in the electrograms appeared. The latency between the A. V. node electrogram (N potential) and the response of the bundle of His (H potential) lengthened progressively during the cooling, until the H potential disappeared (figs. 2 and 3). The N-H latency lengthened 290 por cent of the control value, as an average. The auricle-A. V. node latency (A-N) was lengthened by 14 per

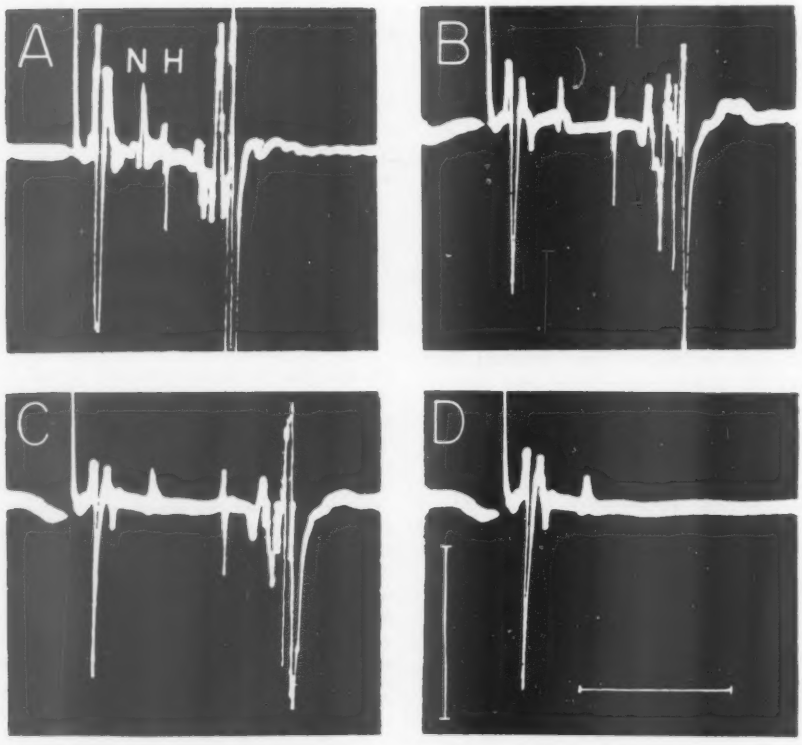

FIG. 2.—*Effect of cooling the A.V. node region. Electrograms recorded from the zone of the A.V. node and the bundle of His of a heart driven at a constant frequency. The first excursion is the stimulus artifact; it is followed by the action potential of the auricle; N, electrogram of the atrio-ventricular node; H, potential from the bundle of His, followed by the ventricular response. A, control; B, C and D 5,10 and 15 seconds after the application of a cold jet of saline solution. Note the lengthening of the N-H latency and the decrease of the amplitud of the N potential. In D the H potential disappeared. Vertical calibration: 8 mV. Time calibration: 180 msec.*

cent as an average. This lengthening reached its maximal value when the N potential disappeared. Prior to its disappearance, a decrease in the magnitude of the N potential was observed (figs. 2 and 3). The same jet of saline solution, applied at 32° C, did not produce any appreciable change.

The amplitude of the N potential can also be modified by changes in the frequency of activation of the A.V. node through the auricle. Progressive increase of the frequency produced reductions of the N potential, while a decrease caused opposite effects (fig. 4).

Adrenaline solutions (1-2 μg in 1 ml) injected to a heart driven at a constant rate, shortened the A-N and N-H latencies and increased the amplitude of the N potential (fig. 5). These effects

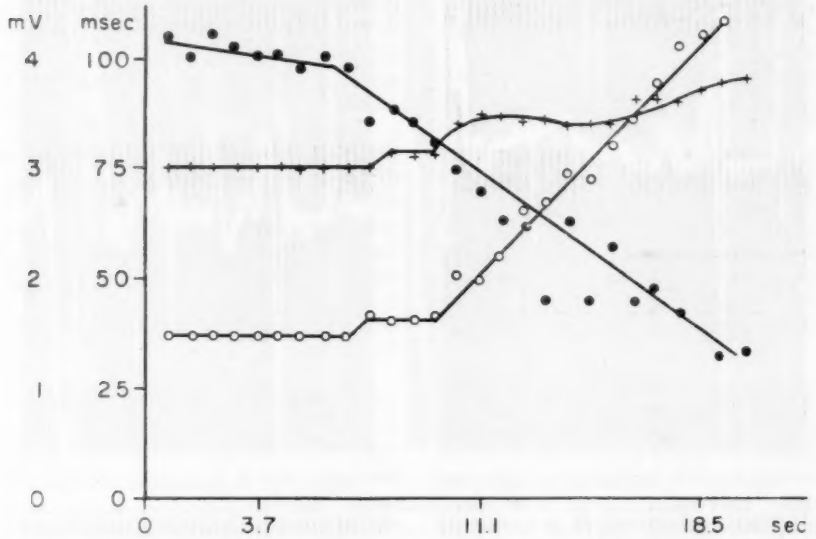

FIG. 3. — *Changes of the N-H (circles) and A-N (crosses) latencies, and of the amplitude of the N potential (dots) produced by cooling the A. V. node region. Abscissae, time of application of cold (seconds). Ordinates, left scale: magnitude of the N potential (mV). Right scale: N-H and A-N latencies (msec).*

were not due to any change in heart rate, since the rate of stimulation was always higher than the maximal rates produced by the adrenaline injection. The N-H latency was shortened to 46 per cent and the A-N latency to 82 per cent of their control values as an average. During the effect of adrenaline, the impulses from the auricle could be propagated to the bundle of His at higher frequencies than was possible without this hormone.

The modifications elicited either by cooling or by adrenaline were always reversible.

DISCUSSION

The functional refractory period. — It has been shown that the N and H potentials represent the activation of the A. V. node and the bundle of His respectively (Alanís, González and López, 1958; Alanís, López, Mandoki and Pilar, 1959). The simultaneous recordings of the action potentials of the auricle, the A. V. node, the bundle of

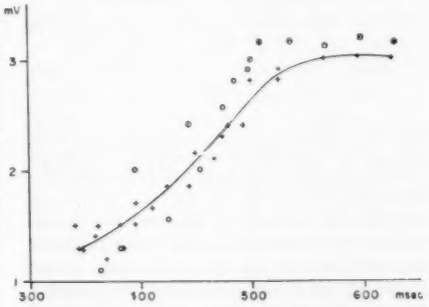

FIG. 4. — *Variation of the amplitude of the N potential produced by the increase (crosses) or decrease (circles) of the frequency of stimulation of the auricle. Abscissae: intervals between stimuli (msec). Ordinate: amplitude of the N potential (mV).*

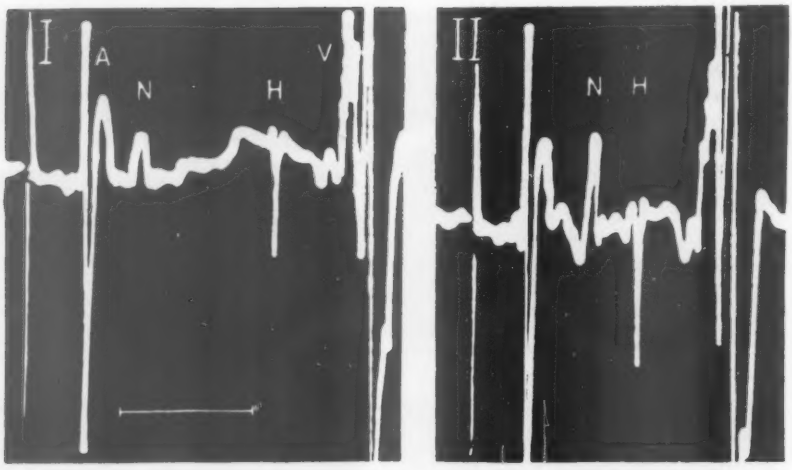

FIG. 5.—*Effects of adrenaline. Stimulation of the right auricle at a constant rate (3.4/sec). Symbols as in fig. 2. In I the N potential appears small and the N-H latency is long. In II during the action of 1 μg of adrenaline, the amplitude of the N potential increases and the N-H latency is shortened. Time calibration 100 msec.*

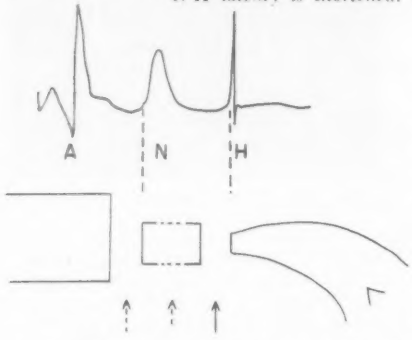

FIG. 6.—*Diagram that shows the probable sites of the specialized conducting system in which propagation of impulses is critical. The electrogram of the auricle (A), the A. V. node (N) and the bundle of His (H) are drawn above. The histological structures activated are represented by the blocks illustrated below. Under all the conditions studied, the junction between the A. V. node and the bundle of His is the most critical region (full line arrow). The other two possible sites for locating the second functional discontinuity, are indicated by the two arrows (broken line); one within the A. V. node and the other in the junction between the atrium and the A. V. node.*

His and the ventricle, made it possible to measure the F. R. P. for the propagation of impulses through the different structures interposed between the auricle and the ventricle.

The F. R. P. for the propagation between the A. V. node and the bundle of His is the longest in the auriculo-ventricular conduction system and corresponds to the F. R. P. of the A. V. propagation. The values of this latter F. R. P. found by Krayer, Mandoki and Méndez (1951) in the heart-lung preparation and Rosenblueth (1958) for hearts *in situ*, differ from those obtained by us. The differences observed can be attributed to differences in temperature. As all tissues were affected by the low temperature of the perfusing blood (32° C), the figures obtained for the different structures are comparable. In a few experiments performed at 37° C, all the values of the F. R. P. diminished, however the F. R. P. of the propagation from the A. V. node to the bundle of His remained the longest.

In a previous paper (Alanís, López, Mandoki and Pilar, 1959) two functional discontinuities were postulated, based among other facts on the existence of two different values for the refractory period, determined by stimulating with progressively increasing frequencies. The shorter value corresponds to the propagation from auricle to the A. V. node and the longer to the propagation from the A. V. node to the bundle of His. The two different values of the F. R. P. determined by pairs of stimuli, give further support to the hypothesis of two functional discontinuities.

The considerable delays of the propagation during the refractory periods would take place primarily in these two critical zones. These delays are not due to low conduction velocity, as has been discussed already by Rosenblueth (1958). This author, using a different experimental method, suggested that the delays were due mainly to stops of the impulses at regions where functional discontinuities exist. Rosenblueth's conclusions are valid when the curve representing intervals between stimuli (abscissae) against the intervals between the corresponding responses, has a horizontal branch, as is the case with the H_1-H_2 and the N_1-N_2 curves (fig. 1).

The H_1-H_2 curve presents in addition in its left segment, an ascending branch (fig. 1). A similar curve was first described by Lucas (1910) in a neuromuscular preparation, which contains a well known histological and physiological discontinuity. This branch is also present in the curve of the auriculo-ventricular propagation of the mammalian heart (Krayer, Mandoki and Méndez, 1951). In this case the impulse travels through several different tissues and one might threfore attribute the left branch of the curve, to the existence of an anatomical discontinuity. However, Forbes, Ray and Griffith (1923) working on a nerve trunk, and Miledi (personal communication) studying single nerve and striated muscle fibres, have observed the existence of the left branch. These results obtained in histologically homogeneous system show that the ascending branch is not a distinctive property of discontinuous structures. The ascending left branch would thus be only an additional indication of a temporary stop of the propagation of an impulse during the refractory period, since for a shorter interval between stimuli, there is an increase of the interval between the corresponding responses.

The effect of cooling the A. V. node region. — The lengthening of the N-H latency and the later disappearance of the H potential as a result of cooling the A. V. node region could be due to a reduction of the excitability of the bundle of His near the A. V. node or to a diminution in the amplitude of the stimulus whereby the A. V. node excites the bundle. There is evidence that cooling reduces the amplitude of the A. V. node potential (figs. 2, 3). If the N potential is the physiological stimulus for the activation of the bundle of His, its diminution would indicate a reduction in the magnitude of the stimulus. On the other hand it is a general observation that cooling of excitable tissues tends to reduce their excitability. Therefore, probably both factors would operate. If the A. V. node cells function as a single unit, the progressive diminution in the amplitude of the N potential produced by cooling, could be due to a delay in the recovery process of the A. V. node, which would then be activated earlier in its refractory period (Hoffman and Suckling, 1954; Alanís, López, Mandoki and Pilar, 1959). If the A. V. node is constituted by individual cells or groups of cells with different duration of their recovery processes, a reduction in the number of cells discharging per impulse could contribute

to the diminution in the N potential. This would imply the alternation of activity in different elements and an alternation in the size of the action potentials. This alternation was not observed.

Modifications produced by adrenaline. – Among all the variables studied until now (Alanís, López, Mandoki and Pilar, 1959), only adrenaline produced a shortening of A-N and N-H latencies. Since this substance enhances several physiological properties of the cardiac tissues, conduction velocity for example, it is not easy to clarify the mechanism whereby these latencies are shortened. The hormone seems not to affect to the same extent the whole conducting system, but has a greater effect on the regions where propagation has a smaller margin of safety (functional discontinuities). Propagation from the A. V. node to the bundle of His appears to be preponderantly affected by the favorable action of adrenaline. The increased amplitude of the N potential produced by adrenaline is in agreement with the similar increase observed by Otsuka (1958) in isolated Purkinje fibers.

The observation that after injections of adrenaline impulses can be propagated at higher frequencies from the A. V. node to the bundle of His, indicates that the corresponding refractory period is shortened by this substance. Krayer, Mandoki and Méndez (1951) reported that small doses of adrenaline, which did not modify the heart rate appreciably, were able to shorten the F.R. P. of the A. V. propagation. Under the present experimental conditions the limiting link of this propagation was the junction between the A. V. node and the bundle of His, as mentioned above, therefore one can reasonably assume that this junction is very sensitive to the action of adrenaline.

Evidence for the functional discontinuities at the A. V. node. – It has been shown previously (Alanís, López, Mandoki and Pilar, 1959) that there are at least two critical regions for the propagation of impulses from the atrium to the bundle of His. The propagation from the A. V. node to the bundle of His is always delayed or blocked first, under the conditions reported elsewhere (Alanís, López, Mandoki and Pilar, 1959) and in the present study. These conditions are the following: a) increase in the frequency of stimulation; b) acetylcholine, ouabaine or acetyldigitoxin injections; c) brief periods of asphyxia; d) cooling of the A. V. node region; e) propagation in incompletely recovered tissues, measured by the application of pairs of stimuli. In contrast to the other factors, adrenaline improved in general the propagation through the regions studied. These variables, although of different nature, all modified predominantly the propagation from the A. V. node to the bundle of His.

In a previous publication (Alanís, López, Mandoki and Pilar, 1959), it was asserted that the refractory period of the A. V. node and of the bundle of His was shorter than the refractory period for the propagation from one of these tissues to the other. The definition of the F. R. P. suggested by Rosenblueth, Alanís and Mandoki (1949) is applicable only to fibers, not to junctions. In the previous and in the present study, the F. R. P. of the bundle of His was determined with electrodes at a certain distance from the A. V. node. Since the diameters of the fibers of the bundle decrease towards the A. V. node, the yuxtanodal region of the bundle has probably the longest refractory period. This region was not explored; it might determine the length of the refractory period for the propagation from the A. V. node to the bundle of His.

Regarding the second critical region

in which impulses are blocked (fig. 6), it has been assumed tentatively to be located at the junction between the atrium and the A. V. node (Alanís, López, Mandoki and Pilar, 1959). Rosenblueth and Rubio (1955) has postulated the existence of several discontinuities within the A. V. node. The simultaneous lengthening of the A-N latency and the reduction in the N potential amplitude observed by us, are compatible with Rosenblueth's hypothesis. However the available evidence appears insufficient to draw definite conclusions, since all these results could be attributed as well to the properties of the junction between the auricle and the A. V. node.

Summary

1. In isolated and perfused hearts of dogs, the electrical activities of the auricle, the A. V. node, the bundle of His, and the ventricle were registered. Steel microelectrodes (4-12 μ) were used, placed in the appropriate regions of the interauricular and the interventricular septa.

2. The functional refractory period (F. R. P.) for the propagation of impulses from the A. V. node to the bundle of His, was 440 msec and 320 msec for the propagation from the auricle to the A. V. node. The F. R. P. for the auriculo-ventricular propagation was always identical to the F. R. P. of the propagation from the A. V. node to the bundle of His. These values were obtained from hearts at 32° C.

3. Cooling the region of the A. V. node showed that the more critical segment for the propagation is the junction between the A.V. node and the bundle of His. Later the propagation from the atrium to the A. V. node was also affected. These changes were evidenced by lengthening of the N-H and the A-N latencies. Simultaneously, a decrease in the amplitude of the N potential was observed.

4. Adrenaline injections shortened the above mentioned latencies, and increased the amplitude of the N potential. The shortening was greater for the N-H latency than for the A-N latency. The changes caused by cooling and by adrenaline were always reversible.

5. The present experimental findings give additional support to the hypothesis postulated previously (Alanís, López, Mandoki and Pilar, 1959) that there exist, at least, two functional discontinuities at the nodal region. One of them is located between the A. V. node and the bundle of His, and is the most critical for propagation. The other lies either within the A. V. node, or at the junction of the auricle and this node.

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ON THE INTEGRATION OF RESPIRATORY MOVEMENTS III. THE FIFTH NERVE AFFERENTS (*)

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In the two previous papers of this series ([5, 6]) emphasis was made on the importance of somatic afferent impulses from several sources for the integration of respiratory activity. With respect to the cranial nerves, the role of the vagal afferents has been fairly well established ([4, 7, 12]). The inhibitory effects mediated through glossopharyngeal afferents are also well known, particularly those involved during deglutition; in this nerve, excitatory as well as inhibitory fibers have been described ([10]).

As regards the fifth nerve, its importance in respiration is almost confined to the knowledge of the sneezing reflex. A closer relation of trigeminal afferents to normal breathing might be suspected on anatomical grounds. The central neurons of the sensory roots of this nerve are in close relation to the reticular activating system of the mesencephalon. Very little, however, has been established on the physiological side, although cyclic respiratory discharges have been recorded at the region of the mesencephalic root of the fifth nerve ([3]).

The present paper deals with some of the effects of direct stimulation of the nasal mucosa and of some of the afferent branches of the fifth nerve upon respiratory muscle's activity.

Methods

Rabbits and cats under light pentobarbital anesthesia were used. A tracheal cannula was inserted low in the neck. The trachea was sectioned above the cannula and tied with a thread, together with the esophagus. The esophagus was also sectioned periferally, and pharynx and larynx were dissected free from the surrounding tissues by cutting all muscles and nerve branches till the base of the skull, which was exposed at the upper posterior wall of the pharynx. The vagus and the glossopharyngeal nerves were usually cut near their emergence at the base of the skull. In many cases the facial and the hypoglossal nerves were also sectioned.

A glass tubing was inserted by a small opening in the posterior upper

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wall of the pharynx. This glass cannula was connected to a rubber tube 1.50 m long which, at the other end, had a mouthpiece with a thermocouple inside it. Blowing by mouth through the mouthpiece passed through the nasal airways of the animal, a current of air at room temperature, because of the length of the tube, and the thermocouple could time precisely the applied stimulus. The air currents could be made to go in the opposite direction by aspirating at the mouthpiece. In this last case, timing of the stimulus was made easier by slightly warming the mouthpiece with the hand. Another way to apply this kind of stimuli was to connect an artificial respiration pump to the system. In some cases the animal was allowed to breathe either through his own nose or through the tracheal cannula properly prolonged by a piece of tubing duplicating the normal dead space and resistance to flow. In some animals, a branch of the fifth nerve, the spheno-palatine, was mounted upon electrodes for stimulation. The stimulator used was a square pulse generator (Grass S4).

Respiratory activity was recorded as electromyograms of different respiratory muscles, *a*) the diaphragm, at the muscle fibers inserting at the xiphoideal appendix, exposed without opening the thorax or the abdomen; *b*) from either the external or the internal intercostal muscles and *c*) from the abdominal muscles, usually the oblique minor. The lead electrodes were small hooks of # 36 nichrome wire soldered to flexible enameled copper wire. The records were taken on a Grass Polygraph after amplification through a Grass E. M. G. and an Integrator preamplifier. In some cases the electromyograms were recorded on a cathode ray oscilloscope (Tektronix dual beam, type 502) and photographed from the screen.

Results

A. The responses to single stimuli. – When a current of air is passed through the nasal path, there is a response involving some changes in muscle respiratory activity. The response is complex and it has at least three components, although they are not always well defined. The first component is inhibition. It is the most constant of the three components (figure 1), it affects either inspiration or expiration depending on what phase of the respiratory cycle the stimulus is applied. Expiratory activity is affected more than the inspiratory (figure 2). The degree and duration of this inhibition are variable depending on several factors. The stronger the stimuli the greater the inhibition, between limits, because very strong stimuli are almost purely excitatory. The effects are greater and the threshold is lower the later in the phase of the respiratory cycle the stimulus is applied. The degree of existing respiratory activity plays a role; depressed respiration is more easily inhibited.

This first inhibition varies from a slight decrease in the number of muscle discharges to complete inhibition lasting from 0.1 to 1 second (figures 1, 2, 3, 8 and 9). Generally, inhibition of a respiratory movement is followed by activity that appears in the antagonistic muscles (figures 2B and 6).

After this initial inhibition there is a period of increased activity which usually is evident till the next complete respiratory cycle (figure 2). In some cases, this excitatory component affects the following antagonistic respiratory movement and, sometimes, the excitation is apparent during the same phase of the cycle in which the stimulus is applied, immediately following the initial inhibition (figures 2 and 6). In opposition with the initial inhibition, the excitation affects mainly, or stron-

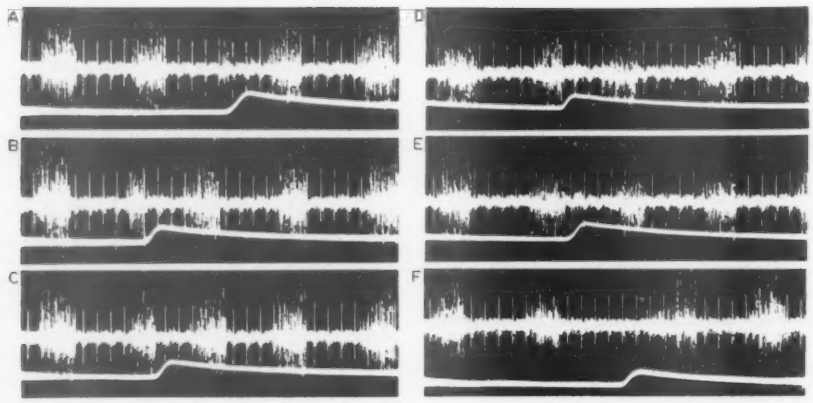

FIG. 1.—*Effects of passing an air current through the nasal airway on inspiratory activity. Rabbit, 7th, 9th and 10 cranial nerves sectioned. Upper record: Electromyogram of the diaphragm. The timing of the stimuli is marked by the thermocouple indicator (lower record). The stimuli applied during inspiration (A to D) inhibits this activity but they can make the next inspiration appear earlier. E. A stimulus applied early in expiration shortened the expiratory phase. F. The stimulus applied late during expiration lengthened the expiratory phase. The following inspiration was enhanced.*

gly, the inspiratory activity. Thus, the excitatory component is very often revealed by a premature or by a deeper inspiration (figures 1, 3, 5 and 7). The excitatory component is very easily demonstrated in hyperventilated animals (figure 8).

Following the excitatory component, there usually comes a residual persistent period of inhibition, which also affects mainly inspiratory activity and produces smaller inspirations, a slower rhythm, or both (figures 5 and 7).

The described responses are of the same type for synergistic muscles, diaphragm and external intercostals (figure 4). In the same manner, the internal intercostals and the abdominal muscles are affected similarly for a given stimulus. The effects are seen irrespective of the direction of air flow through the nose (figure 3). Similar results were obtained by tactile stimulation by touching the nasal mucosa with a thread or a small cotton wick.

The threshold for these responses is very low. Two parameters are important in determining the effectiveness of such stimuli, one is the amount of air, the other, the most important one, is the velocity of flow. The threshold value was roughly obtained by using an artificial respiration pump of the Starling type, with a stroke volume of 25 ml with a rate of 20 per minute. In this case the velocity of flow is sinusoidal. The threshold is lower with faster rates of flow. A continuous flow of air gives a change in respiration only at the beginning, or when there is a sudden change of rate in flow. It is interesting to note that in most cases the volume and rate of flow of the air breathed by the animal during quiet respiration are enough to induce changes in respiratory activity (figure 4). Above threshold, the responses appear more constantly, the initial inhibition is more evident and the residual facilitation or inhibition are more

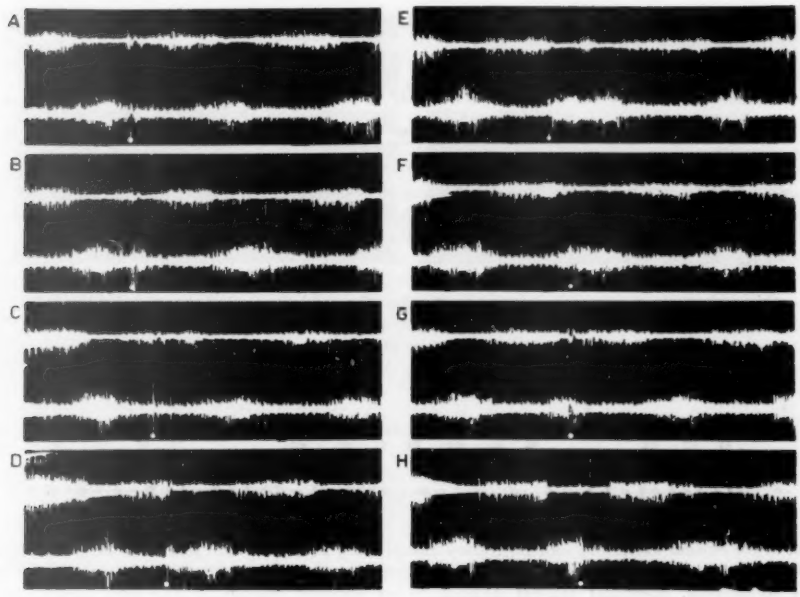

FIG. 2. — *Effects of stimulating the nasal airway on inspiratory and expiratory muscle activity. Cat, 9th and 10th cranial nerves sectioned. Electromyograms of an abdominal muscle (oblique minor), upper record, and of the diaphragm, lower record. Dots mark the time of application of the air current (stronger stimuli than in figure 1). There is an immediate inhibition of expiratory activity when the stimuli are applied during this phase. If applied during inspiration, inhibition of this activity is less evident, except when the stimuli are applied during the second half of this phase of the respiratory cycle (compare A and F with G and H). During the first half of inspiration the response may be almost purely excitatory (B, D and E).*

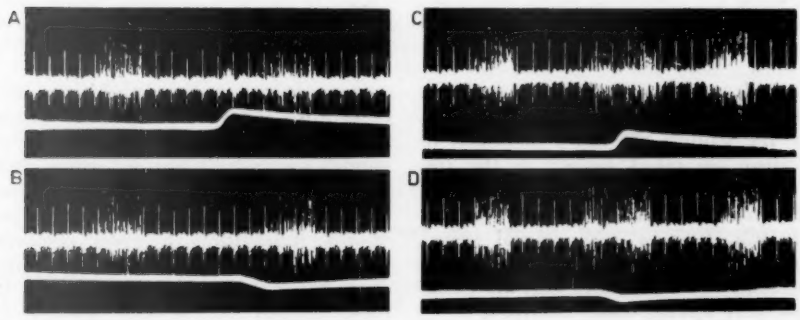

FIG. 3. — *Similarity of the results of blowing (A and C) and of aspirating air (B and D) through the cannula inserted at the upper part of the pharynx. Rabbit, electromyogram of the diaphragm. The lower record marks the timing and polarity of the stimuli. A and B, during quiet breathing. C and D, animal with increased dead space.*

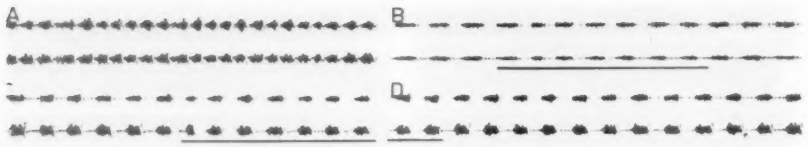

FIG. 4. — *Similar responses recorded on two different inspiratory muscles. Rabbit, 7th, 9th, and 10th cranial nerves sectioned. Electromyograms of external intercostal, upper record, and of the diaphragm, lower record. In A, the two dots, mark the time of application of a sudden current of air through the nose. In B, the horizontal lower line, marks the period during which the animal was breathing through his own nose, without variation of the dead space or resistance to the air flow. Note the greater effects at the beginning. In C, the animal was breathing through the tracheal cannula. During the period marked by the horizontal line, the nasal airway was ventilated with an artificial respiration pump with about the same stroke volume and rate of the animal's own breathing. D, is the end of the period which started in C.*

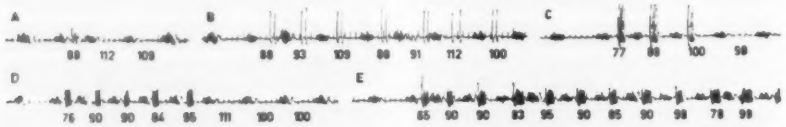

FIG. 5. — *Residual excitatory and inhibitory components modifying the successive responses seen on inspiratory activity. Rabbit, 7th, 9th and 10th cranial nerves sectioned. Electromyogram of the diaphragm. Electrical stimuli applied to an afferent branch of the fifth nerve. The artifacts are seen in the records. The figures below each expiratory phase give the duration of the respiratory cycle in per cent of the average controls. A. A short train of stimuli applied during expiration shortens that expiratory phase, but the next one is prolonged and there is a reduction of the following inspiratory activity. This shows a residual excitation lasting during one respiratory cycle and a residual inhibition which is apparent for at least the following two cycles. B. The effects of the stimuli (two shocks) applied every cycle interfere with each other. The excitatory component may, approximately, cancel the inhibitory one due to the previous stimulus. At the following cycle, the excitation elicited may fail to show due to the summated inhibition of the two previous trains of electric shocks. C. By increasing the number of electric shocks in the train, the excitatory component seems to be more marked. In D the frequency of stimulation was 20 per second, and of 50 per second in E.*

marked and long lasting (compare figures 1 and 2). Furthermore, the stronger the stimuli the more easy it is to produce an effect earlier in the inspiratory or the expiratory phases of the cycle (figure 10). Sections of the 7th, 9th, 10th or 12th cranial nerves do not appreciably modify the responses, although when both vagi are intact they may appear more complex.

B. The results of repetitive stimulation. — Generally, if a second stimulus is applied at the next cycle, the responses obtained are less clear. This is true mainly for the inhibition that constitutes the first component of the responses (figure 7). In a series of stimuli successively applied every cycle their effects are progressively decreasing. In some cases it is possible to obtain indefinitely a good response to each stimulus if these are applied every other cycle (figure 10B), but in most animals that is only possible by stimulating every five or six respiratory cycles. A peripheral factor seems to be a factor in this behaviour, since sometimes the responses to successive stimuli

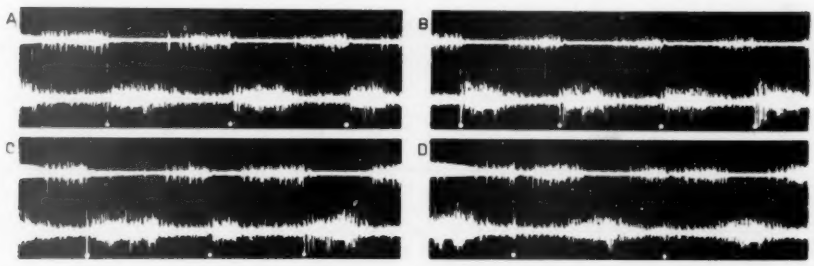

FIG. 6. — *Effects of the stimulation of the nasal airway by an air current applied every cycle (dots). Cat, electromyogram of the abdominal muscles, upper record, and of the diaphragm, lower records. 9th and 10th cranial nerves sectioned. A. Stimuli applied at the end of the expiratory phase. At the start of the series. The stimuli inhibits expiration and initiates the inspiration. In the successive cycles there is a progressive reduction of expiratory activity. B. Later in the series. The summated inhibition is shown by a reduction of inspiratory activity and shortening of this phase. C. Still later in the series, the stimuli do not produce the same effects every cycle. D. The stimuli applied at the middle of the expiratory phase inhibits expiration which again reappears sometimes less, sometimes more active, resulting in cycles of varying duration.*

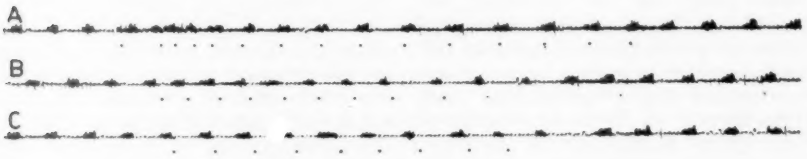

FIG. 7. — *Long lasting effects of repeated stimulation. Rabbit, 7th, 9th and 10th cranial nerves sectioned. An air current is passed through the nose at the moments marked by the dots. Electromyogram of the diaphragm. A. The initial inhibition produced in the inspiratory activity by the first stimulus is less marked and appears irregularly with the following ones. After the last stimulus in the series there is evidence of persistent excitation. B. Residual inhibition becomes more and more prominent during the series. There is also evidence of final persistent excitation. C. The time elapsed between B and C (one minute) was not enough to dissipate completely a state of inhibition which is evidenced by the new applied stimuli.*

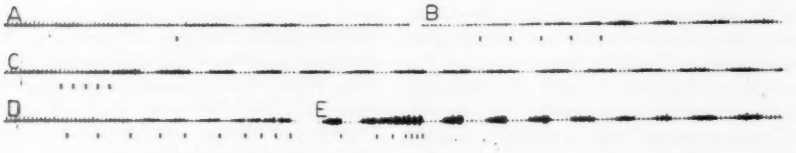

FIG. 8. — *Excitation of inspiratory activity by afferent stimulation. Rabbit under hyperventilation by artificial respiration that was maintained at a constant level throughout this observation. 9th and 10th cranial nerves sectioned. Electromyograms of the diaphragm. Stimulation of the nasal airway by air currents at the moments signaled by the bars below. A to D, increasing the number or the frequency of the applied stimuli. E, shows the end of the stimulation period started in D. Note the tendency to rhythmic breathing which might not be disturbed by the stimuli.*

FIG. 9. — *Rhythmic breathing facilitated by afferent stimulation of the nasal airway by air currents. Rabbit, electromyograms of the diaphragm. A. Both vagi were cut at the neck, with the animal under artificial respiration. The apneusis induced was turned to rhythmic breathing by the stimulus signaled by the thermocouple record below. B. After hyperventilation, and following suppression of artificial respiration, rhythmic breathing appeared "spontaneously". This rhythmicity was facilitated by afferent stimulation as in C.*

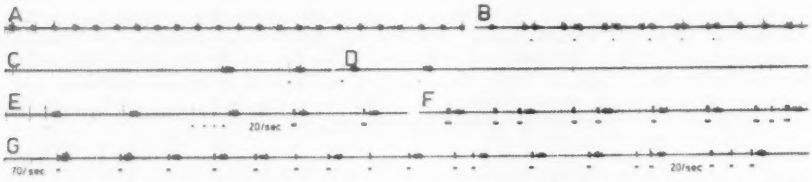

FIG. 10. — *Inspiratory activity induced by afferent electrical stimulation to a branch of the fifth nerve. Rabbit, electromyograms of the diaphragm. A. Control. B. Single shocks (dots) inducing an early inspiratory movement at a short interval. After B, breathing was stopped by an overdose of pentobarbital. Single shocks again induce inspiratory movements (C), which do not appear if the stimuli are discontinued (D). E to G, the rate of breathings is a function of the number of shocks applied. E, single shocks during the first half of the record, and trains at 20 per second at the end (last two horizontal lines). F, the same trains at 20 per second. G, trains at 70 per second, excepting the last three which are at 20 per second.*

may appear unchanged by alternating blowing and aspiration of air.

As for the residual excitatory and inhibitory parts of the responses, the effects of repeated stimulation are different. They do not tend to attenuate with successive stimuli. On the contrary, they tend to summate and give a complex picture due to mutual interaction (figures 6 and 7). Another factor which may add to the complexity of the responses to repetitive stimulation, is the fact that ventilation may be altered and other compensatory mechanisms come into play.

Summation of excitation not only occurs by the repeated application of the same stimulus (figure 8), the excitatory component may be also enhanced by previous or simultaneous stimulation of other somatic afferents, as by compression of the thoracic or the abdominal walls, or merely by tactile stimulation of the belly or the legs. This last facilitatory effect is mainly seen on expiratory activity. The summated residual effects, either excitatory or inhibitory may persist for relatively long time, up to several minutes (figure 7).

C. Fifth nerve afferent stimulation and rhythmic breathing. — By section of both vagi and under properly adjusted artificial respiration, it is possible to stop rhythmic breathing having a continuous inspiratory activity of the diaphragm. In this condition, a current of air through the nose inhibits that

activity, and if the stimuli are applied at a frequency near that of normal respiration it is possible to induce rhythmic breathing which tends to persist after removing the stimuli, although with progressively increasing duration of the cycles (figure 9A). If the animal is hyperventilated and then the artificial respiration is stopped, the animal, under increasing asphyxia, may eventually show "spontaneous" rhythmic breathing. This appearance may be accelerated by afferent stimulation (figures 9B and C).

Rhythmic breathing may be induced not only by inhibiting a basal continuous activity. In hyperventilated animals, inspiratory movements may appear as a result of fifth nerve afferent stimulation. In this case the excitatory component increases as the number of stimuli are increased, and there is evidence that the persistent excitation do come alternating with inhibition in a cyclic manner (figure 8). When breathing is stopped by an overdose of pentobarbital, the same kind of stimuli, or the electrical stimulation as well, may induce the reappearance on inspiratory activity. In this last case, the stronger the electrical shocks or the greater the number of them in a volley, the faster can be made the rate of breathing (figure 10).

Discussion

It is evident from the presented results that the fifth nerve afferents have an influence on respiratory activity. Their importance may be considered with regard to their effects upon normal respiration and their possible role in the determination of rhythmic breathing.

1. The effects upon normal respiration. — It has been shown that stimulation of the nasal mucosa gives rise to complex responses: inhibition followed by residual and persistent excitation and inhibition. The excitatory component seems to be less evident with weak stimuli, such as those due to the passage of air through the nose during the normal respiratory act. This might explain why inhibition is the dominant effect in this case (figure 4). There is another reason to expect an apparent small effect of naturally occurring afferent stimulation of the trigeminal territory, i. e., the observation of the lesser effects of successive stimuli. This phenomenon, not considering the possible peripheral factor due to accommodation of receptors, is commonly seen as part of the normal activity of the mesencephalic reticular formation. It is due to interaction between afferent volleys with each other and with corticofugal projections ([8]). In this particular case two other factors seem to be also important: *a*) The fact that during the period of summated residual inhibition elicited by the previous stimuli, the excitation may fail to show (figure 7). *b*) The possible alteration of ventilation which may change the level of central excitability in the opposite direction. In summary, the role of the fifth nerve afferents would be to contribute to the smoothness of the respiratory movements, sharing this action with other afferents particularly the vagal ones.

2. The determinants of rhythmic breathing. — The alternation of excitation and inhibition as a result of stimulation of fifth nerve afferents, suggests the possibility that this mechanism may play a role in determining rhythmic breathing. Perhaps this mecnanism does not have much importance during quiet breathing. It would be obscured by other far more important driving forces, for example, the vagal afferents.

The results presented demonstrate the presence of cyclic changes in excitability of the respiratory integrating

centers. Stimuli are more effective in inhibiting respiratory activity when they are applied at the late part of the inspiration or the expiration (figures 2, 8, 9). Similar results were described by Larrabee and Hodes [10] by stimulation of the superior laryngeal nerve. These authors suggested the existence of a "naturally" occurring central inhibitory state increasing progressively during inspiration, and they assumed that the inhibition produced by afferent stimulation would cause addition to the naturally-occurring central state, at the level of the expiratory center. The similarity of the present results with those of Larrabee and Hodes would call for a similar explanation. There is, however, a difficulty, because these authors did not record expiratory activity. Since afferent stimulation has an inhibitory action on expiratory activity (figures 2 and 6), the hypothesis of Larrabee and Hodes can not be applied to the present results. Rhythmic breathing was explained by Pitts, Magoun and Ranson [11] by a periodic inhibition of the inspiratory center coming from the pneumotaxic region through the expiratory center. The possibility of having simultaneous activity of inspiratory and expiratory muscles [5], and the fact that both activities may be inhibited by afferent stimulation (figures 2 and 6) induce us to look for another mechanism of rhythmic breathing. Cohen and Wang [3] have described many groups of neurons in the pons of the cat, which show respiratory cycle activity. Brodie and Borison [2], based mainly on ablation experiments, have assigned to the pons the function of respiratory "modulator".

Accepting this recent evidence of the pontile reticular formation as an important part of the integrating mechanism of rhythmic breathing, what would be the role of afferent impulses? We could think, with Larrabee and Hodes, that the effects of afferent stimulation would only reveal the existence of cyclic changes in excitability occurring "naturally" in those last structures. But we could also present the hypothesis that afferent impulses might be the cause of those changes. In support of this hypothesis we have the following facts: *a*) Afferent impulses from many sources have an effect on respiratory activity. *b*) The threshold for these effects are usually very low. *c*) A great proportion of the afferent stimulation occurs naturally in a cyclic manner. They can be put into activity during normal breathing, by the passage of air through the airways; by the inflation and deflation of the lungs; by the movements of the thoracic and abdominal walls; by the stretching of some respiratory muscles [6]. *d*) Stimulation of a single afferent path may have marked influence on respiratory activity. Stimulation of two or more of them may summate their effects. *e*) Suppression of the vagal influence may stop rhythmic breathing. Deafferentation of the spinal cord makes this stop more likely to occur [6]. *f*) Breathing stopped by an overdose of barbituric may be made to reappear by afferent stimulation [1] (figure 10). *g*) After hyperventilation, respiratory movements can be induced by afferent stimulation (figures 8 and 9). *h*) The persistent effects of afferent stimulation [5, 9, 10], and the cyclic nature of its prolonged action (figures 7, 8 and 9).

All this evidence supports the idea that rhythmic breathing would be the result of afferent influences acting upon an integrating mechanism. The integrating mechanism, the modulator of Brodie and Borison [2], would drive a final common path, the medullary centers, whose continuous activity would be induced by the carbon dioxide of arterial blood.

Summary

In cats and rabbits under pentobarbital anesthesia, the changes of the activity of respiratory muscles were recorded when currents of air were passed through the nasal cavity and upon electrical stimulation of a branch of the fifth nerve. The responses obtained consisted of inhibition followed by excitation and inhibition of long duration (figures 1, 3, 6). The last two components of the responses may appear simultaneously or somewhat alternatively in time (figures 7, 8, 9). They are susceptible of summation (figures 6, 7). The threshold for responses is very low, the passage of air through the nose during quiet breathing is enough to induce changes (figure 4). In general, expiratory activity is more easily inhibited than the inspiratory one (figure 2). On the contrary, inspiratory activity is more easily excited.

During apneusis produced by double vagotomy, afferent periodic simulation may elicit rhythmic respiratory movements by periodically inhibiting inspiratory continuous activity (figure 9). Under hyperventilation, afferent stimulation can also induce rhythmic breathing by producing short periods of inspiratory activity (figure 8). Breathing stopped by an overdose of pentobarbital may be induced again by afferent stimulation (figure 10).

The possible importance of fifth nerve afferent stimulation in normal breathing, and in the determination of rhythmicity is discussed. During normal quiet breathing, the role of the fifth nerve afferents would be to contribute to the smoothness of the respiratory movements. In determining rhythmic breathing the importance of the fifth nerve afferents would be, along with the effects of other afferent impulses from several sources, to make the activity of the central integrating neurons of a cyclic nature.

Acknowledgement

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ALGUNAS CARACTERÍSTICAS DEL REFLEJO DEPRESOR DE ORIGEN CORONARIO (*)

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En una publicación anterior (González y Erlij, 1958) se describió un reflejo depresor circulatorio que se origina en el sistema coronario. Al ocluir el seno venoso coronario en corazones de perros, hay caída de las presiones arteriales sistémica y pulmonar y bradicardia. La frecuencia respiratoria no se modifica. El efecto depresor desaparece al seccionar los vagos. El estímulo que lo desencadena actúa sobre presorreceptores localizados en el sistema vascular de la coronaria izquierda.

El presente trabajo fué hecho con el objeto de estudiar algunos de los factores que influyen sobre este reflejo y precisar la localización de los receptores.

Método

Los experimentos fueron hechos en perros y gatos. Como los hallazgos fueron semejantes en ambas especies, la descripción se limitará a los resultados obtenidos en los primeros. Como anestésicos se usaron el pentobarbital (30 mg/kg de peso, por vía intraperitoneal), cloralosa (70 mg/kg por vía intravenosa) o hidroxidiona sódica [Viadril (1), 100 mg/kg por vía intravenosa]. Después de abrir el tórax bajo respiración artificial se disecó el nervio depresor en su porción intratorácica en el punto en que sus dos ramas se unen al tronco del vago izquierdo. Una vez que se había inyectado un anticoagulante (heparina 2.5 mg/kg como primera dosis y 10 mg cada 45 minutos), se canuló la vena yugular izquierda. Se introdujo a través de la orejuela derecha una cánula de Morawitz al seno venoso coronario (fig. 1), conectándola con la cánula de la yugular izquierda y con un manómetro de mercurio. Al cerrar la pinza P se registraba la presión del interior del seno venoso. Al iniciar los experimentos se infló ligeramente el globo de hule de la cánula de Morawitz para evitar la salida de sangre entre las paredes del seno y las de la cánula.

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(1) Agradecemos a los Laboratorios Pfizer de México el obsequio de la hidroxidiona sódica empleada en estos experimentos.

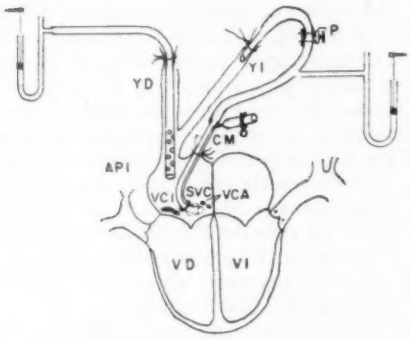

FIG. 1. — *Esquema de la técnica usada. Para aumentar la presión del sistema vascular coronario se ocluye con la pinza P el tubo de hule que va de la cánula de Morawitz, CM, a la cánula de la yugular izquierda, YD: yugular derecha, canulada para el registro de la presión auricular. SVC: seno venoso coronario con la cánula de Morawitz. VCA: venas cardíacas anteriores. VI y VD: ventrículos izquierdo y derecho. VCI: vena cava inferior.*

La frecuencia cardíaca se registró mediante un par de electrodos, uno en aurícula y otro en ventrículo, conectados a un electroencefalógrafo modelo III-A de Grass. La medida de la presión arterial se hizo de una arteria femoral con un manómetro de mercurio.

La otra arteria femoral se seccionó y se conectaron cada uno de sus cabos a los extremos de la rama horizontal de una cánula en T. La rama vertical estaba en conexión con una bomba de perfusión con débito constante. Ocluyendo el cabo central de la arteria o la rama vertical de la cánula se podía irrigar la pierna con solución salina proveniente de la bomba o restituir la circulación normal. Entre la bomba y la arteria femoral se intercaló un manómetro de mercurio, que registraba los cambios de resistencia periférica de la pierna, mientras se perfundía ésta con solución salina. Para evitar el exceso de este líquido dentro del organismo, la perfusión sólo se establecía unos segundos antes de evocar el reflejo y se suspendía unos segundos después.

Al final de cada experimento se disecó el seno venoso coronario para comprobar si la colocación de la cánula de Morawitz había sido hecha correctamente.

RESULTADOS

A. *Acción de los anestésicos.* — De los anestésicos empleados el que más deprimió las respuestas reflejas fué el pentobarbital sódico. Con este barbitúrico las caídas de presión eran generalmente pequeñas y la bradicardia refleja poco ostensible o nula (fig. 6-I). Cuando las dosis eran mayores que 30 mg/kg, el reflejo se presentó sólo ocasionalmente. Con la cloralosa la aparición del reflejo depresor fué más uniforme y su magnitud era mayor. La bradicardia refleja sin embargo era nuevamente irregular en su aparición. Con dosis mayores de 70 mg el reflejo depresor desapareció. Los mejores resultados se obtuvieron con la hidroxidiona sódica. El reflejo se observó con caídas de presión arterial marcadas. La bradicardia refleja se manifestó casi invariablemente (21 veces en 22 experimentos). Cuando sobre la cantidad habitual de hidroxidiona se inyectó un décimo más de la dosis, las respuestas reflejas se deprimieron, pero a los cinco minutos eran de una magnitud igual a la que tenían antes de inyectar el excedente.

B. *Efecto de la oclusión del seno venoso.* — Al ocluir el tubo de hule que iba de la cánula de Morawitz a la yugular con la pinza P se registraba la pre-

sión intrasinusal. Esta maniobra produjo un aumento de presión dentro del seno venoso (fig. 2).

Las presiones máximas que se obtuvieron dentro del seno venoso al ocluirlo por completo fluctuaron entre 33 y 60 mm de Hg. Se podían obtener presiones mayores cerrando la pinza P e inyectando con una jeringa rápidamente solución salina isotónica. Para atenuar los aumentos de presión dentro del seno, no se ocluía por completo su conexión con la yugular; se disminuía parcialmente la luz del tubo de hule que unía al seno con la vena yugular con la pinza P. De esta manera se podía analizar la relación que hay entre la presión intrasinusal y la magnitud del reflejo.

C. *Efecto del aumento de la presión intrasinusal.* — Cuando el intervalo entre dos respuestas reflejas sucesivas era de dos minutos o más, la segunda respuesta no se modificó. Este fue el lapso mínimo con que se evocaban las respuestas en los experimentos. Como puede verse en la figura 2, mientras mayor la presión intrasinusal, mayor es la bradicardia e hipotensión reflejas. Las magnitudes de la hipotensión y de la bradicardia, medidas como por ciento de sus valores basales, aumentan al crecer la presión dentro del seno (figs. 2, 3, 4). La respuesta refleja empezó a manifestarse cuando la presión dentro del seno era aproximadamente de 10 mm de Hg. A partir de esta cifra los efectos reflejos ascendían en forma de S itálica, para acercarse a un valor máximo (fig. 3). Este valor varió en cada animal; se alcanzó aproximadamente cuando la presión intrasinusal era de 37 a 55 mm de Hg (fig. 3). En algunos experimentos se elevó dicha presión hasta 85 mm de Hg. Todas las observaciones se hicieron aumentando primero las presiones dentro del seno y disminuyéndolas después.

D. *Influencia de los depresores y los nervios del seno carotídeo.* — Los reflejos que compensan las variaciones de la presión arterial ejercen una acción antagónica sobre el reflejo depresor coronario. Para atenuar este efecto se cortó el nervio depresor izquierdo y se ligaron ambas carótidas primitivas. La bradicardia y la hipotensión aumentaron, la primera entre 6 y 9 % y la segunda entre 10 y 19 %. Las respuestas reflejas comenzaron a manifestarse con valores menores de presión dentro del seno venoso (fig. 4).

E. *Bradicardia y resistencia periférica.* — En algunos experimentos, sobre todo en los que se utilizó el pentobarbital, el reflejo depresor apareció sin bradicardia (fig. 5-I). En los experimentos en los que hubo tanto bradicardia como hipotensión, al inyectarle atropina (0.25 mg/kg, por vía intrave-

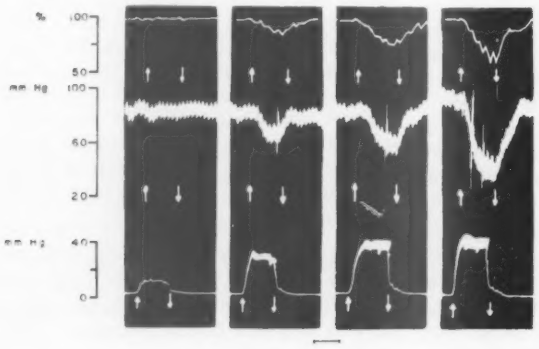

FIG. 2. — *Influencia de la presión intrasinusal sobre la magnitud de las respuestas reflejas. De arriba a abajo: frecuencia cardíaca, presión arterial sistémica y presión intrasinusal. La frecuencia cardíaca en esta figura y las que siguen está dada en tanto por ciento de las cifras basales. Entre las flechas, oclusión de la salida del seno venoso. Señal de tiempo: 20 segundos.*

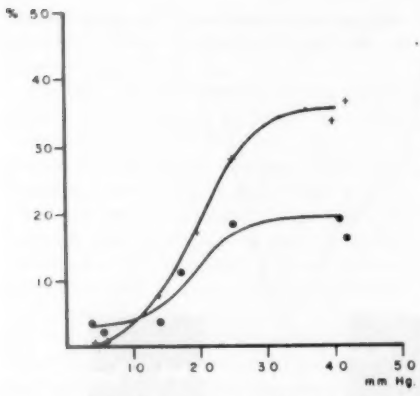

FIG. 3. — *Relación entre la presión intrasinusal y la magnitud de los efectos reflejos. Puntos, bradicardia refleja; cruces, hipotensión refleja. Ordenadas, efecto reflejo en porciento de la basal. Abscisas, presión intrasinusal.*

nosa) desapareció la bradicardia y persistió la caída de la presión arterial (fig. 5-II). Evitando así la disminución de la frecuencia cardíaca, la hipotensión fue 10 a 27 % menor que cuando había bradicardia (fig. 5-II b y c).

Cuando se ocluyó el seno venoso disminuyó la presión en el manómetro colocado entre la bomba de perfusión (véase método) y el cabo periférico de una arteria femoral (fig. 6). Al mismo tiempo había una caída paralela de la presión arterial sistemática. Esto se observó antes y después de las inyecciones de atropina.

Discusión

Influencia de los anestésicos. Las respuestas reflejas, bradicardia e hipotensión, son susceptibles de ser deprimidas fácilmente por los anestésicos, ya que dosis ligeramente mayores que las usadas las hacen desaparecer. De los anestésicos empleados el que da mejores resultados es la hidroxidiona sódica, luego la cloralosa y por último el pentobarbital sódico. Estos hechos concuerdan en cuanto a la cloralosa y nembutal con lo que sucede en otros reflejos reguladores de la presión arterial (Alvarez-Buylla, 1950).

El estímulo. Según Dawes y Comroe (1954), a partir de las terminaciones aferentes coronarias, sólo han sido bien establecidos diversos quimiorreflejos del

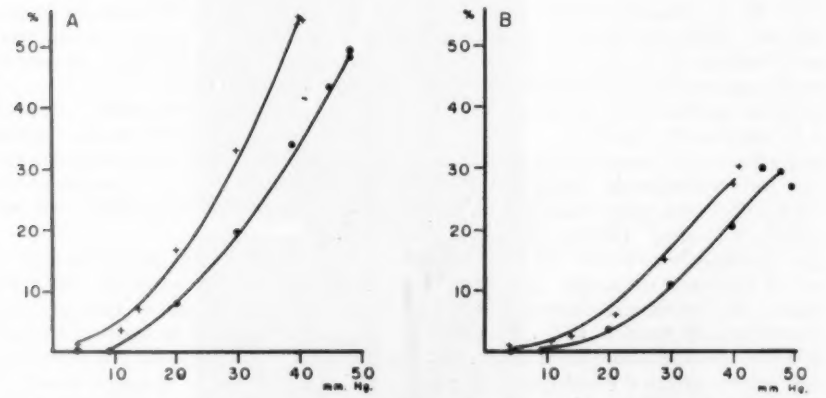

FIG. 4. — *Influencia de los presorreceptores sobre la magnitud de los efectos reflejos. A, hipotensión refleja; B, bradicardia refleja. Los puntos corresponden a las curvas obtenidas antes de seccionar el nervio depresor aórtico y ligar ambas carótidas primitivas; las cruces, después de esta maniobra. Ordenadas y abscisas igual que en la figura anterior.*

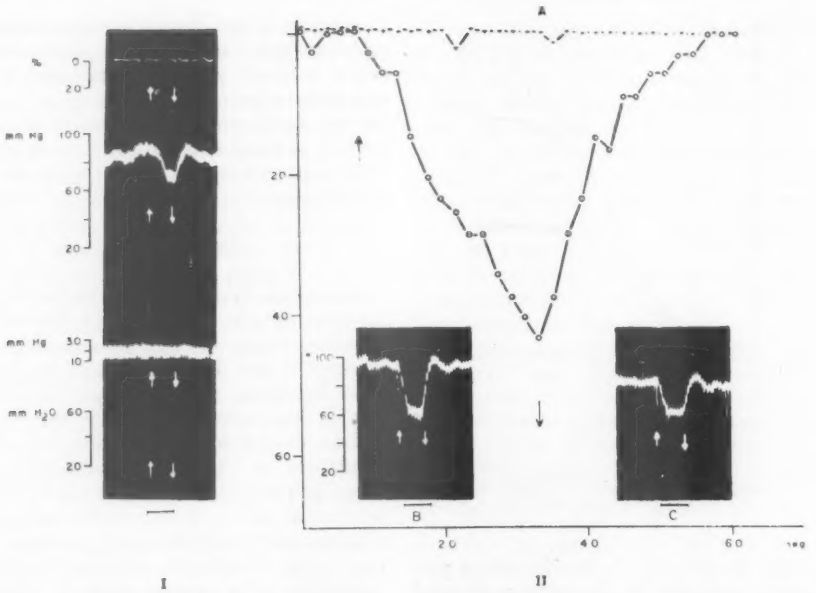

FIG. 5. — *Independencia de la bradicardia y de la hipotensión reflejas. I. Hipotensión refleja sin bradicardia. De arriba a abajo, frecuencia cardíaca, presión arterial sistémica, presión pulmonar y presión venosa. II. Frecuencia cardíaca refleja antes de atropinizar (círculos) y después de atropinizar (cruces). B y C, hipotensión refleja antes y después de atropinizar al animal. Flechas y señales de tiempo como en la figura 2.*

tipo de v. Bezold-Jarisch (Jarisch y Richter, 1939). En nuestros experimentos el reflejo se despierta en el momento en que se ocluye el seno venoso. Esta oclusión produce un aumento de presión dentro del territorio venoso que desemboca en el seno, lo que concuerda con los resultados de Gregg y Dewald (1936). Por otra parte, Gregg y Shipley (1947) y Gregg (1950), encontraron que durante la oclusión del seno venoso, el volumen de sangre que fluye a través de las arterias coronarias sólo disminuye de un 8 a 10 %. Verdun y Ramírez de Arellano (1958) observaron este mismo efecto y concluyeron que se debe a una vasodilatación coronaria refleja. Esto nos permite concluir que el reflejo coronario descrito en el presente trabajo tiene su punto de partida en presorreceptores, descartando la posibilidad de que se origine en quimiorreceptores, ya que el flujo coronario prácticamente no varía.

Estos receptores responden a aumentos de presión en el territorio venoso coronario y la magnitud en hipotensión y bradicardia reflejas son proporcionales a la magnitud de presión intra-sinusal.

Localización de los presorreceptores. En el momento de ocluir la salida del seno venoso es probable que hay un aumento de presión en todo el sistema coronario. La bradicardia e hipotensión reflejas empiezan a aparecer desde el momento de la oclusión (fig. 2). Estos dos hechos nos hacen pensar que los presorreceptores coronarios responden a aumentos de presión. la bradicardia e

hipotensión reflejas aumentan paralelamente con los aumentos de presión en el seno (figs. 2, 3, 4).

La hipotensión sistémica desencadenada por el reflejo produce una disminución importante de la presión y del flujo coronario (Gregg, 1950; Eckenhoff y col., 1947 y Eckenhoff y col., 1948). Pero la variación de presión en las arteriolas y capilares que están incluídas en el músculo cardíaco podría aumentar por la mayor resistencia que se opone al flujo sanguíneo al cerrar el seno y por el efecto de bomba secundaria que hace el miocardio con el masaje sistólico. Algún tiempo después de la oclusión el flujo coronario vuelve a adquirir un valor semejante al que tenía antes (Gregg y Dewald, 1936; Verdun y Ramírez, 1958). Este hecho y la disminución de la presión de perfusión de las coronarias por la caída de la presión sistémica (Gregg, 1950; Eckenhoff y col., 1947 y 1948) sugieren que si bien en el momento de la oclusión aumentó la presión en todo el sistema coronario, posteriormente debió caer del lado arterial, en tanto que del lado venoso continuó elevada. Por lo tanto, si los receptores se hallaran del lado arterial cesarían de responder y la bradicardia y la hipotensión serían transitorias.

Las cifras tensionales del territorio

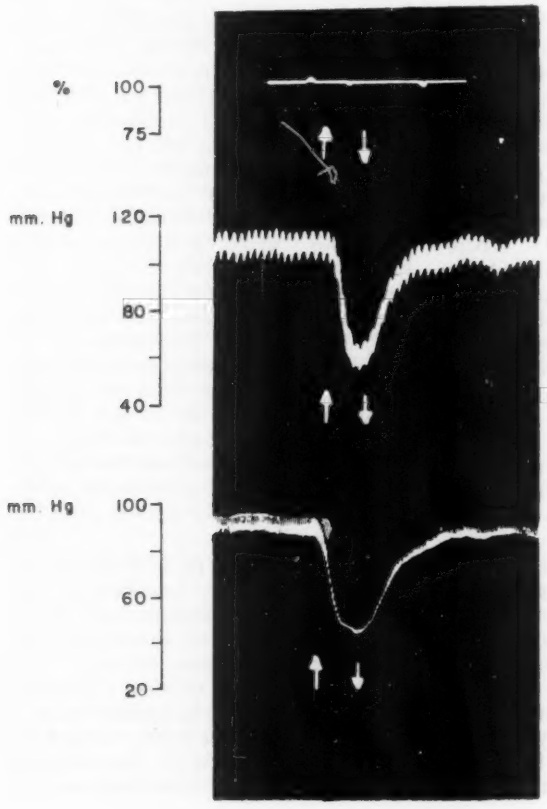

FIG. 6. — *Modificación refleja de la resistencia periférica. De arriba a abajo frecuencia cardíaca, presión arterial sistémica y presión arterial en una extremidad inferior perfundida. Señal de tiempo y flechas, igual que en la figura anterior.*

venoso dentro de las que se activan los presorreceptores (5 a 55 mm de Hg) están por abajo de las de la presión arterial mínima. En nuestros experimentos esta última nunca fue inferior a 70 mm de Hg. Concluímos por lo tanto, que es en el territorio venoso del seno coronario donde se encuentran estos receptores.

Cuando el globo que tiene en su punta la cánula de Morawitz y que está dentro del seno venoso es inflado, éste ejerce presión contra las paredes del seno. Aunque esta presión esté dentro de las cifras en que se despiertan las respuestas reflejas, no se observa hipotensión ni bradicardia reflejas. Por lo que no parece ser éste el sitio del territorio venoso en que se localizan los presorreceptores.

Los componentes del reflejo. Los compontes de la respuesta refleja, hipotensión y bradicardia, son independientes, ya que el reflejo puede manifestarse con hipotensión y sin bradicardia. Además, esta última es más lábil a la acción de los anestésicos. Estos hechos confirman la disociación funcional de los centros cardioinhibidor y vasodilatador, observada al estimularlos por otras vías (Alvarez-Buylla, 1949).

La hipotensión refleja depende de la bradicardia refleja en un 10 a 25 %, que es el porcentaje en que disminuye la caída de presión arterial al atropinizar al animal cuando el reflejo se presenta tanto con disminución de frecuencia como con hipotensión. En los experimentos en que se irrigó un miembro posterior con la bomba de perfusión quedaba excluído el efecto de la presión arterial del resto del cuerpo. Durante el desencadenamiento del reflejo, la presión del líquido de perfusión disminuyó. Puesto que la irrigación se hacía con un flujo constante, este descenso indica un abatimiento de la resistencia periférica por vasodilatación. Esta vasodilatación es la responsable de la hipotensión durante el reflejo coronario cuando el animal se halla atropinizado.

Resumen

Se estudiaron algunas de las características del reflejo depresor coronario descrito por González y Erlij (1958). Los experimentos se hicieron en perros y gatos con resultados semejantes. Como anestésicos se usaron pentobarbital, cloralosa o hidroxidiona sódica (Viadril). Las respuestas reflejas varían de acuerdo con el anestésico empleado; siendo de mayor magnitud con la hidroxidiona y menores con cloralosa y pentobarbital.

Los receptores en que se inicia el reflejo son del tipo de los presorreceptores. Oclusiones parciales del seno venoso coronario producen aumentos proporcionales de la presión en su interior. Las respuestas reflejas obtenidas aumentan mientras mayor sea la magnitud de la presión dentro del seno (figs. 2 y 3). La ligadura de las carótidas primitivas y el nervio depresor aórtico hacen que la respuesta refleja sea mayor y que empiecen a manifestarse con presiones intrasinusales menores (figura 4). Puesto que la presión arterial coronaria disminuye después de la oclusión del seno y las cifras de presión intrasinusal dentro de las que se despierta este reflejo están por abajo de la mínima arterial, se concluye que los presorreceptores se encuentran localizados en el territorio venoso de la coronaria izquierda.

Los componentes de la respuesta refleja, hipotensión y bradicardia, son independientes. En algunos animales la presión arterial cae sin disminución de la frecuencia cardíaca. En otros con ambos componentes reflejos, al inyectar atropina desapareció la bradicardia permaneciendo la hipotensión, aunque de menor magnitud (fig. 5). Las medidas de la resistencia periférica indican que

la hipotensión es debida a vasodilatación.

Summary

Some of the properties of the coronary depressor reflex described by González and Erlij (1958) were studied. Experiments, using dogs or cats lead to similar results. The anesthetics used were pentobarbital, chloralose or sodium hydroxydione (Viadril). The size of the reflex response depends on the anesthetic employed; being greatest with hydroxydione, less with chloralose and least with pentobarbital. The receptors from which the reflex originates are of the pressoreceptor type. Partial occlusions of the venous coronary sinus produced increases of the pressure to the degree of occlusion. The reflex responses were greater when the pressure inside the sinus was higher (Figs. 2 and 3). The ligature of the common carotids and the severance of the left aortic depressor nerve favours the reflex response, inducing it with slighter increase of the intrasinusoidal pressure (Fig. 4). Since the coronary arterial pressure diminishes when the sinus is occluded, and the intrasinusoidal venous pressure increases, it is concluded that the pressoreceptors are found on the venous side of the left coronary. This fact is even more substantiated, if it is taken into account that the reflex is produced with increases of the venous intrasinusal pressure, the values of which are always below the sistemic diastolic pressure.

The different components of the reflex response: hypotension and bradicardia are independent. In some animals the systemic arterial pressure dropped, while the cardiac rate did not. In others with both reflex responses present, bradicardia disappeared after atropine injection, the hypotension remaining, although of a lessened degree (fig. 5). Peripheral resistance measurements suggest the hypotension to be due to vasodilatation.

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RESPUESTA EMOCIONAL CONDICIONADA Y DROGAS PSICOTRÓPICAS (*)

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UNA de las principales metas de la Psicofarmacología es controlar la ansiedad conociendo los mecanismos que se ponen en juego para conseguir tal fin bajo la acción de los medicamentos. Buscando estos mecanismos Miller et al. (19) demuestran que con cloropromacina la disminución del temor motivado por evocación condicionada, "no es atribuible a efectos motores y/o sedativos de la droga"; por su parte Weiskrantz (25) sugiere la hipótesis que la falta de reactividad y de atención de los animales que han recibido reserpina, a pesar de que con dosis altas muestran al EEG una excitación central (16, 21), se debe a bloqueo de la información sensorial que reciben; y John et al. (14) por trabajos en gatos, proponen la teoría de que la acción descondicionante de la reserpina se debe a una interferencia en las asociaciones aprendidas.

Para valorar el estado de ansiedad, autores como Masserman (18), Jacobsen (12) y Norton (10), han ideado sistemas de calificación para el comportamiento de los animales durante las pruebas experimentales; otros han realizado estudios tendientes a establecer el significado de la defecación o respuesta emocional vegetativa (REV) de ratas colocadas en situaciones especiales: Hall, en 1934 (12), hace observaciones sobre la REV de animales colocados en un ambiente extraño (open field) sin hacer intervenir ningún estímulo condicionado; Brady y Hunt, en 1951 (4), proponen la técnica de la REC ("anxiety") controlando principalmente la REV de ratas condicionadas a un estímulo acústico que evoca un shock eléctrico; Broadhurst, en 1957-58 (5, 6, 7) adopta la técnica de Hall y revisa la validez de la REV como índice del comportamiento emocional; Ryall, en 1958 (22), con igual método, trata de valorar la acción de algunos psicofármacos, y más recientemente Taeschler y Cerletti (23, 24) han considerado la importancia de este dato en una serie de trabajos donde buscan establecer la relación entre acción inhibidora de la REV y actividad moto-

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ra en las ratas, e índice terapéutico de un tranquilizante.

Durante el desarrollo de un vasto estudio en curso que persigue el perfeccionamiento de una clasificación farmacológica de los psicotrópicos, nos ha parecido conveniente tomar en consideración estas observaciones y desarrollar una técnica, originada en la respuesta emocional condicionada de Brady-Hunt (REC), perfeccionada en los detalles de ejecución, para tener un test de base preciso y reproducible en lo posible.

Material y métodos

Animales. — Ratas albinas machos de 60 a 80 días de edad.

Aparatos. — Una caja de madera cuya base mayor es de 40 cm por 24 cm y con 16 cm de altura; sus paredes superior y anterior son de plexiglás y el piso está formado por una reja metálica conectada a un circuito eléctrico. En una de las paredes hay un generador acústico capaz de emitir un número determinado de sonidos por unidad de tiempo. Esta caja-reja se coloca en un compartimiento especial que la aísla parcialmente del ambiente exterior; un espejo permite la observación del animal.

Procedimiento. — Los experimentos siempre se llevaron a cabo en el mismo ambiente (una pieza tranquila pero sin suprimir los ruidos normales de un laboratorio), y fueron conducidos por la misma persona. Se desarrollaron según el siguiente programa:

1er. día: Adaptación: cada rata permanece 10 a 15 m en la caja-reja, sin recibir alguna clase de estímulos.

2º a 5º días: Condicionamiento: cada animal tiene una sesión diaria de 15 a 20 m, durante la cual se hace dos veces la presentación de la situación condicionante; ésta es: 3 m de un estímulo acústico —ruido que se repite 16 veces por segundo— reforzado justamente al comienzo y al final por un shock eléctrico con corriente de 2 a 5 m A, 120 volts y 50 ciclos.

6º y 7º días: Readaptación o purificación de la respuesta: dos veces diarias, cada animal tiene una sesión de 15 a 20 m en la caja-reja, durante la cual no recibe estímulo alguno.

8º día: Descanso.

9º día: Control: durante una sesión de 10 m a cada animal se le presenta el estímulo condicionado una vez y por 3 m en esta oportunidad acompañado solamente del shock final.

10º día: Prueba farmacológica: durante una sesión de 10 m se presenta una vez y por 3 m el estímulo condicionado; los animales se han dividido en grupos de 3: un grupo sirve de control y los otros han recibido diferentes dosis del producto por estudiar, por vía subcutánea. Cada animal pasa solamente una vez por la prueba farmacológica.

Una vez individualizada la dosis capaz de inhibir la REV en la mayoría de las ratas de un lote, se averigua si el producto examinado tiene de por sí alguna actividad sobre la motilidad intestinal, para esto se hace la prueba de la comida con carbón ([23]) según el siguiente plan: a ratas que han estado 24 h en ayunas se les suministra por vía oral una suspensión de carbón; algunas de estas ratas sirven de control y las otras han recibido 40 m antes (70 en el caso de la imipramina) el producto por vía subcutánea. Veinte minutos después de la comida con carbón las ratas se sacrifican y se diseca su intestino a fin de medir la longitud total a partir del píloro, y la distancia que ha avanzado el medio de contraste. La comparación se hace en base a la relación porcentual de estas dos medidas. Como controles se emplearon cinco animales; igual número para cada producto, a excepción de la benacticina donde se controló con 8 ratas cada dosis.

Datos. — Los datos que se valoran son: 1) REV (defecación); 2) CM (comportamiento motor), que es la actividad motora desarrollada durante la permanencia del animal en la caja-reja; 3) resistencia y agresividad de los animales durante las operaciones de traslado entre caja-reja y jaula-hogar; 4) actitudes presentadas por las ratas calificándolas según el esquema de Jacobsen [13]: muy tenso (iiii), moderadamente tenso (iii), ligeramente tenso (ii), actitud de relajación (i). Los demás signos del "fear syndrom" [17] no se observaron constantemente, por lo cual no se anotan.

Observación de adiestramiento

Se observó en un grupo de ratas su comportamiento y la ordenación de la REV durante cada una de las sesiones enumeradas: En el 1er. día la REV se presentó en el 60 % de los animales durante los primeros minutos de permanencia en la caja-reja, lo que se observa frecuentemente al colocar las ratas en un ambiente nuevo.

En el 2º día de prueba, 1º del condicionamiento, sólo en el 10 % hubo REV al colocarlos en la caja-reja; la REV fué general después del primer shock eléctrico y durante todo el tiempo siguiente hasta el fin de la sesión, pero sin ordenación relativa a los períodos de shock-sonido o silencio. El 2º día del período de condicionamiento, en todos los animales hubo REV antes del primer shock, la REV después de él es mayor y se repite con cada nuevo shock. En los días 3º y 4º de este mismo período la REV estuvo presente en todos los animales tan pronto entraron en la caja-reja, y después del primer shock comienza a ordenarse dentro de los intervalos limitados por 2 shocks y ocupados por sonido. De la actitud iii del 1er. día del condicionamiento y que apareció después del primer shock, se ha llegado a una actitud iiii permanente durante cada sesión y a un estado de agresividad tal que se ha hecho necesario el empleo de guantes de cuero para manejarlos. Las 4 sesiones del período de readaptación se realizan para que los animales pierdan la situación de ansiedad (observable como REV) que se les presenta con el solo hecho de entrar en la caja-reja. En la primera de estas sesiones la REV se presentó en todos y fué notoria la actitud iiii. Este estado desapareció gradualmente, y en la cuarta sesión los animales ya caminan por la caja-reja o quedan en su puesto pero en actitud i ó ii y solamente en un 5 % de ellos se presentó la REV.

La prueba de control se hace para saber cuántos animales han adquirido la REC; esta es la aparición de la REV con la presentación de la señal acústica. Se rechazan aquellos en los cuales la REV aparezca antes del comienzo del estímulo condicionado o no aparezca durante este y antes del shock.

Es de anotar que el estado de agresividad y resistencia a las manipulaciones aparecido durante el período de condicionamiento, no desaparece ni disminuye. Estos animales se continuarán observando.

Resultados

Cloropromacina. — La prueba se realizó 1 h después de la inyección; las dosis empleadas fueron de 0.1, 0.2, 0.5, 1, 2, 3, 5 y 10 mg/kg. En los animales tratados con 10 mg/kg y en uno de los tratados con 5 mg/kg no hubo REV, a la vez se observó, sólo en ellos, un estado de quietud en las operaciones de transporte entre la caja-reja y la jaula-hogar; en comparación, los demás animales mostraron el mismo comportamiento agitado de los controles y defecaron. Además, durante la permanencia en la caja-reja y la presentación del estímulo condicionado, pudo verse un notorio estado de postración con inmo-

Acción de los agentes psicotrópicos sobre el comportamiento y la respuesta emocional en ratas condicionadas

		Respuesta Emocional Condicionada (REC)		Comportamiento Motor (CM)	Actitud	Resistencia y Agresividad
		REV, dosis mínima inhibidora mg/kg.	Test de la comida con carbón. %. Lim. de confianza ($P < 0.05$)	— inhibido + activado ○ no modificado (en relación con los controles)	iiii muy tenso iii moderadamente tenso ii ligeramente tenso i actitud de relajación	+ presente — ausente
Neurolépticos (8) Descondicionantes de indiferencia (10) Tranquilizantes mayores (26)	Clorpromacina	10	17.77 42.63	—	i	—
Desconfusionantes (1) Agentes estimulante-depresores	Imipramina 2169 I. S.	50 50	38.86 49.54 28.33 46.05	○ —	ii i	+ —
Antifóbicos (1) Drogas psicotrópicas anti ACh. (26) Descondicionantes de disociación (10)	Benacticina	2 a 5	53.96 64.34 47.95 65.69	+	i	+
Relajantes (1) Tranquilo-sedativos (26) No descondicionantes (10)	Meprobamato	100	44.30 70.38	—	i	—
	Controles		46.85 77.69		iiii	+

vilidad pasiva (i) a diferencia de los controles que mostraron una inmovilidad de alarma (iiii).

En el test de la comida con carbón se aprecia una diferencia significativa ($P < 0.05$) entre los tratados con 10 mg/kg y los controles, indicando una disminución de la motilidad intestinal con esta dosis.

Meprobamato. — La prueba se realizó 1 h después de la inyección; las dosis empleadas fueron de 20, 50 y 100 mg/kg. Las ratas tratadas con 50 mg/kg presentaron una disminución de la actividad espontánea; sin embargo en las operaciones de cambio de lugar mostraron la misma agitación de los controles tratando de escapar como éstos y defecando abundantemente durante el período condicionado. Con 100 mg/kg los movimientos torpes y no hubo REV con la presentación del estímulo acústico.

En el test de la comida con carbón no hay diferencia significativa ($P < 0.05$) entre los animales tratados con 100 mg/kg y los controles.

Benacticina. — La prueba se realizó 1 h después de la inyección; las dosis empleadas fueron de 1, 2, 5, 10, 20 y 100 mg/kg. Con 100 mg/kg, los animales estaban excitados en alto grado, con el sonido condicionado corrieron vivazmente dentro de la caja-reja. Con 50 mg/kg el estado es similar pero en grado algo menor; con dosis de 5 a 20 mg/kg las ratas ocuparon todo el tiempo del experimento inspeccionando atenta y continuamente la caja-reja. El número de ratas tratadas con 2 y 5 mg/kg se aumentó a 7 para cada dosis, obteniéndose el siguiente resultado respecto a la inhibición de la REV: con 5 mg/kg solamente un animal defecó y con 2 mg/kg 3 ratas defecaron con la presentación del estímulo condicionado.

En el test de la comida con carbón no hay diferencia significativa ($P < 0.05$) entre los animales tratados con 2 y con 5 mg/kg y los controles.

Imipramina. — La prueba se realizó 1 ½ h después de la inyección; las dosis empleadas fueron de 20 y 50 mg/kg. Hubo inhibición de la REV en los animales tratados con 50 mg/kg y en uno de los tratados con 20 mg/kg.

En el test de la comida con carbón no hay diferencia significativa ($P < 0.05$) entre los animales tratados con 50 mg/kg y los controles.

2169 I. S. — La prueba se realizó 1 h después de la inyección; las dosis empleadas fueron de 10, 20 y 50 mg/kg. En los animales tratados con 50 mg/kg y en uno de los tratados con 20 mg/kg no hubo REV como sucedió en los restantes tratados con 20 y 10 mg/kg y en los controles. En todas las ratas tratadas se observó posición de relajamiento muscular con extremidades en hiperabducción e hiperextensión, dejando el abdomen apoyado directamente sobre el piso.

En el test de la comida con carbón hay una pequeña diferencia significativa ($P < 0.05$) entre los animales tratados con 50 mg/kg y los controles.

Discusión

Los resultados obtenidos empleando la técnica de la respuesta emocional condicionada (REC) nos parecen dignos de atención porque han confirmado precedentes publicaciones (1, 2, 3, 8, 10) sobre la especificidad de la acción de los varios medicamentos conocidos como tranquilizantes, y los mecanismos que se han considerado en juego.

En efecto se ha visto:

a) la benacticina provoca inhibición de la respuesta emocional vegetativa (REV) con dosis que aumentan el comportamiento motor (CM).

b) el meprobamato y el 2169 I. S. provocan inhibición de la REV con dosis mayores de las que disminuyen el CM.

c) la cloropromacina provoca inhibición de la REV con dosis iguales a las que disminuyen el CM.

d) la imipramina provoca inhibición de la REV con dosis que no alteran el CM.

Se ha completado el estudio de la REC con el test de la comida con carbón donde se ha visto que las dosis capaces de inhibir la defecación no tienen influencia sobre la motilidad intestinal por cuanto concierne a la benacticina, el meprobamato y la imipramina. Una cierta inhibición del peristaltismo se ha notado en las ratas tratadas con 10 mg/kg de cloropromacina y con 50 mg/kg de 2169 I. S., dosis 10 veces mayores que las necesarias para descondicionar ratas de la misma cepa en la prueba del Pole-climbing-response ([10, 11]). Este hecho parece explicable si se tiene en cuenta que el PCR es un fenómeno reflejo, elemental, de carácter obligatorio y automático ([10]), mientras que la defecación emocional significaría una respuesta más natural ya que no es aprendida por la experiencia.

Estas constataciones confirman la oportunidad de desarrollar un examen de los fármacos psicotrópicos comparando varias técnicas, porque sólo de esta manera se puede llegar a una diferenciación entre ellos y racionalizar un empleo selectivo en la clínica.

El aislamiento parcial del ambiente en que se realizó la prueba, ofrece una situación más fisiológica ([15]) que el aislamiento total a la vez que amortigua los estímulos fuertes del mundo exterior y favorece la uniformidad en la obtención de los datos.

La importancia del análisis de los diversos datos indica que la valoración de la respuesta de ansiedad no puede hacerse con uno solo; es necesario complementar la observación de la REV con la observación del comportamiento del animal.

Si se considera un solo dato (REC) la benacticina sería el tranquilizante más potente, pero en la terapéutica de las psicosis graves la cloropromacina ocupa un primer puesto.

Tal vez las dosis de benacticina empleadas en la práctica médica sean algo bajas, ya que son aproximadamente 1/50 de las dosis efectivas en animales, mientras que para la cloropromacina la relación aproximada es de 1/5.

Resumen

En ratas condicionadas para una respuesta emocional obtenida con técnica cuidadosa, se han estudiado cinco drogas psicotrópicas. Se ha conseguido la inhibición de la REV con todos los productos, pero empleando dosis mayores a las comúnmente usadas en la experimentación psicofarmacológica; solamente con la benacticina fueron suficientes dosis bajas.

La REV se ha mostrado independiente del comportamiento motor CM puesto que es posible inhibir la REC con activación del CM.

Si se relaciona la defecación con emotividad y viendo la independencia entre REV y acción sedativa, se concluye que los efectos antiemotivos y sedativo de un mismo producto, pueden ser distintos.

Con benacticina, la disociación entre CM y REC, se observó también entre PCR y CM.

Hay un cierto paralelismo entre inhibición de la REV y descondicionamiento a la PCR, pero con diferencia cuantitativa en la proporción de la dosis.

Summary

A study has been made of the effect of five psychotropic drugs on rats conditioned to furnish a conditioned emotional response under carefully controlled conditions.

Using doses larger than those commonly employed in psychopharmacological testing, an inhibition of the vegetative emotional response (defecation) could be achieved with all compounds tested. Only benactyzine produced inhibition at low doses.

The vegetative emotional response could be shown to be independent of the motor behavior since it is possible to inhibit the conditioned emotional response with an activation of the motor behavior.

Relating defecation to emotivity and given the absence of a relationship between the vegetative emotional response and sedative action, it can be concluded that the antiemotional and sedative effects of the same compound may be separate.

With benactyzine, the lack of an existing relationship between the motor behavior and the conditioned emotional response was equally observed between the pole climbing response and the motor behavior.

There is a certain parallelism between inhibition of the vegetative emotional response and deconditioning to the pole climbing response. Quantitative differences were, however, observed as a function of the doses employed.

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FREE NUCLEOTIDES IN NORMAL AND LEUKEMIC BLOOD CELLS (*)

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FREE nucleotides have been demonstrated in normal and leukemic blood [23] as well as in red blood cells from human beings [22].

In our laboratory we have confirmed the presence of free nucleotides in normal blood cells and in samples from patients with chronic granulocytic leukemia [18, 19].

Besides the adenine nucleotides, AMP, ADP and ATP already reported [22, 23] we have been able to identify the two uridine nucleotides UDPAG and UDPG as well as DPN, TPN, GTP and UTP in both types of blood samples. Other workers have identified some of these substances in normal red blood cells [4, 8, 12, 14].

The isolation and identification of some of these compounds from leukemic blood samples has not been previously reported.

MATERIAL AND METHODS

Blood samples were obtained by venipuncture, collecting directly into ice cold centrifuge tubes. Differential white and red blood cells counts were carried out on separate samples. Heparin was used as anticoagulant (Liquemine Roche, 0.1 ml/40 ml of blood).

The samples were centrifuged immediately at 2000 RPM for 10 minutes in the cold (0-4° C), the plasma was discarded [23] and the blood cells were frozen at −70° C in an alcohol— solid CO_2 mixture, until required.

The methodology for the separation of the nucleotides has been described previously [17], but in this case, the perchloric extracts after neutralization with 2N KOH in the cold, were lyophilysed until ¼ of their original volumes. In this way contamination of the samples by K Cl O_4 was avoided.

In addition to the usual methods for the identification of nucleotides by paper [20] and column chromatogra-

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Abbreviations: DPN and TPN, di- and triphosphopyridine nucleotide; AMP, ADP and ATP, adenosine, mono-, di- and triphosphate; UDPAG, uridine diphosphoacetyl glucosamine; UDPG, uridine diphosphoglucose; GTP, guanosine triphosphate; UTP, uridine triphosphate; P-O-P, inorganic pyrophosphate.

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phy ([6]), purine and pyrimidine ([24]) phosphorus ([11]) and sugar analysis ([1]), enzymatic methods were used ([9, 13]) for identification of compounds such as ADP, ATP, UTP, UDPG and UDPAG, based on the following chemical reactions:

$$\text{A) UDPG} + \text{P-O-P} \xleftarrow{\text{enzyme "1"}} \text{UTP} + \text{glucose-1-PO}_4$$

$$\text{B) UTP} + \text{ADP} \xrightarrow{\text{enzyme "2"}} \text{ATP} + \text{UDP}$$

$$\text{C) ATP} + \text{glucose} \xrightarrow{\text{enzyme "3"}} \text{ADP} + \text{glucose-6-PO}_4$$

$$\text{D) glucose-6-PO}_4 + \text{TPN} \xleftarrow{\text{enzyme "4"}} \text{6-PO}_4\text{gluconic ac.} + \text{TPNH}$$

It has been possible to follow the reduction of TPN (which indicates specifically and stoichiometrically the amount of 6-PO_4gluconic acid formed from the components present in the incubation mixture) by measuring the optical density change with a Beckman spectrophotometer at λ 340 mμ. The conditions for the incubation mixture have been established before ([13]).

In reaction *A* the nucleotide UDPG, was converted in the presence of P-O-P, by an enzymatic extract prepared from rat liver nuclei, into UTP and glucose-1-PO_4. It was then possible to follow the reaction in two directions: from UTP through equations *B* and *C* to the end of equation *D* and, secondly, from glucose-1-PO_4 converted directly to glucose-6-PO_4 by the enzyme phosphoglucomutase (enzyme "5"); glucose-6-PO_4 was converted to 6-PO_4gluconic acid in the presence of TPN by addition of a dehydrogenase as in equation *D*.

For the particular case of UDPAG it was possible to follow the formation of UTP spectrophotometrically ([13]). The acetylglucosamine-PO_4 formed in this reaction can not be detected by this method.

UTP, ADP and ATP have been demonstrated spectrophotometrically ([3, 9, 10, 13]) starting from the adequate incubation mixtures, such as in equations *B* or *C* and adding the lacking factors when necessary.

Enzymatic preparations ("1"). The uridyl transferase ([15]) used in equation *A* has been prepared from rat liver nuclei in accord with the method of Mills, Ondarza and Smith ([13]).

("2") The nucleoside diphosphokinase ([3, 10]) was prepared from pigeon breast muscle by the method of Krebs and Hems ([10]).

("3") The hexokinase was a preparation obtained from yeast by the method of Berger, Slein, Colowick and Cori ([2]).

("4") The glucose-6PO_4 dehydrogenase has been obtained from rat mammary gland, as a mitochondrial preparation in accord with the method of Glock and McLean ([7]).

("5") The phosphoglucomutase was extracted from rabbit muscle and purified by the method of Najjar ([16]).

In the above enzymatic reactions, the enzyme preparations have been checked for contamination with other activities. In every case, to discard the possibility of error, a blank estimation was carried out.

The nucleotides utilised as standards have been purchased from Nutritional Biochemicals, Cleveland, Ohio, excepting UDPAG and UDPG, which were prepared from yeast by the method of

TABLE I

Characteristics of normal and leukemic blood

	NORMAL BLOOD						LEUKEMIC BLOOD		
	S1	S2	S3	S4	S5	Average ± s	L1	L2	Average
VOLUME ml	40	52	35	34	97	—	40	40	—
HEMOGLOBIN g/100 ml .	17.1	19.6	16.1	16.5	18.1	17.5 ± 2.82	7.0	6.8	6.9
HEMATOCRIT %	50	52	49	49	55	51.0 ± 5	20	32	26
LEUCOCYTES/mm³	4.9×10^3	9.4×10^3	8.0×10^3	5.2×10^3	9.7×10^3	$7.44 \times 10^3 \pm 4.69$	231×10^3	305×10^3	268×10^3

Note: White blood cells counts have been carried out in triplicate by the usual clinical laboratory methods.
s = standard deviation.

TABLE II

Nucleotides distribution in normal and leukemic blood

			NORMAL BLOOD						LEUKEMIC BLOOD		
SOLVENT	PEAK	IDENTITY	N1	N2	N3	N4	N5	Average ± s	L1	L2	Average ± s
I	B	DPN	—	1.17	0.56	0.61	0.55	0.73 ± 0.50	1.92	0.57	1.24
	C	AMP	—	1.96	1.46	1.87	2.06	1.84 ± 0.43	2.10	3.07	2.60
II	D	TPN	—	1.08	0.58	0.77	0.62	0.76 ± 0.37	0.69	0.72	0.70
	E	ADP	—	10.96	9.86	10.42	8.40	9.91 ± 1.73	8.40	6.85	7.42
III	XY	UDPAG	—	1.20	—	—	1.40	1.30 ± 0.20	1.30	1.25	1.27
IV	X_2	UDPG	—	1.30	—	—	1.07	1.17 ± 0.23	2.40	3.60	3.00
V	X_4	ATP	42.30	44.38	40.20	38.00	54.22	43.77 ± 10.81	52.00	56.52	54.26
VI	N	GTP	0.52	2.20	0.95	0.80	1.72	1.24 ± 1.41	6.75	7.15	6.95
	O	UTP	0.57	2.20	0.54	0.76	1.44	1.10 ± 1.41	3.00	4.23	3.62

Note: The concentrations of the nucleotides are given in μM/100 ml of blood.
s = standard deviation.

Caputto, Leloir, Cardini and Paladini [5] and from liver by Smith and Mills [21].

Results

Preliminary studies [18, 19] showed the presence in normal and leukemic blood, of the following nucleotides: UDPAG, UDPG, ATP, GTP and UTP. Because some of them appeared to be increased in amount in leukemic blood cells, it was of interest to obtain quantitative results in the nucleotide content of these two types of blood.

The pertinent data concerned with five normal and two leukemic blood samples appear in table I.

With the above samples from table I, the nucleotides were separated by column [6] and paper chromatography [20].

The results of two typical samples of normal and leukemic blood appear on figure 1.

Coenzyme I and II. — DPN and TPN appeared in the position represented in the diagram of elution as peaks B and D, respectively (see figure 1). To obtain a better separation of these nucleotides by paper chromatography, the ascending solvent of Krebs and Hems [10] was utilised. After elution of the spots from the paper chromatogram, with 1 M KCN, these nucleotides were identified by the spectra of their CN-complexes which have a maximum at λ 327 mμ. To calculate their concentration in blood, the molecular extinction coefficient of 18.7×10^3 at λ 260 mμ, was used.

Adenine nucleotides. — AMP, ADP and ATP have been identified by: a) their position in the column after elution with the solvents I, II and V respectively (see figure 1). (In figure 1, AMP corresponds to peak C, ADP to peak E and ATP to X_4). Preliminary experiments with known nucleotides and columns of the same size, were carried out. b) after hydrolysis of the nucleotides with 2 N HCl for 1 hr at 100° C, the purine base was separated by paper chromatography by the method of Wyatt [24]. c) paper chromatography in ethanol / ammonium acetate [20]. d) absorption maximum at λ 257 mμ at pH 2. e) enzymatic technique, ADP by the nucleoside diphosphokinase reaction [3, 10] and ATP by the hexokinase reaction [9].

The molecular extinction coefficients at pH 2 and λ 257 mμ utilised here,

ELUTION DIAGRAMS OF NUCLEOTIDES FROM LEUKEMIC AND NORMAL BLOOD

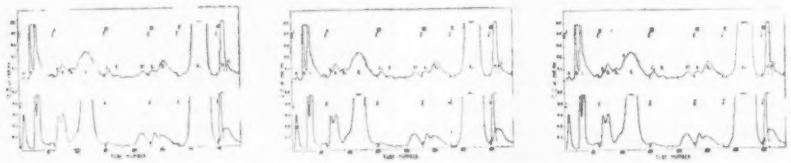

FIG. 1. — *The elution diagram corresponds to λ 260 mμ absorbing material separated with columns of Dowex 1 Cl' x 8 of 1.2 x 20 cm. The eluates were collected in a LKB automatic fraction collector in volumes of 5 ml/tube. The solvents utilised were: I. 0.005 N HCl; II. 0.01 N HCl — 0.01 M NaCl; III. 0.01 N HCl — 0.03 M NaCl; IV. 0.01 N HCl — 0.05 M NaCl; V. 0.01 N HCl — 0.10 M NaCl; and VI. 0.01 N HCl — 0.50 M NaCl. The upper part of the figure corresponds to the perchloric acid soluble nucleotides from a 40 ml sample of leukemic blood (see L2, table I and II). The lower part of this figure represents the nucleotides from a 97 ml sample of normal blood (see N5, table I and II).*

are: AMP $=$ 15.1 $\times$ 10^3, ADP $=$ 14.9 $\times$ 10^3 and ATP $=$ 14.7 $\times$ 10^3.

Uridine nucleotides. – From the elution with solvents III and IV, five peaks were obtained from normal and leukemic blood (see figure 1). The peak XY corresponds to UDPAG and peak X_2 to UDPG as was shown by: a) spectrophotometric characteristics of uridine nucleotides, with absorption maxima at pH's 2 and 11 of λ 260-62 mμ. b) identical Rf values with standard preparations obtained from yeast (5) and rat liver(21) and separated by paper chromatography in ethanol/ammonium acetate (20). c) hydrolysis with 2 N HCl for 1 hr at 100° C, gives uridylic acid from both nucleotides, which was chromatographically separated using the isopropanol/HCl solvent (24). d) sugar analysis after hydrolysis with 0.01 N HCl for 15 minutes at 100° C. The sugars were separated by paper chromatography in ethanol/ammonium acetate (20). e) enzymatic identification as uridine derivatives, with uridyl transferase from rat liver nuclei (13).

The molecular extinction coefficient at pH 7 and at λ 262 mμ of 10.45 $\times$ 10^3, has been taken into consideration for these nucleotides.

Guanosine and uridine triphosphates. – From the elution with solvent VI, two peaks were obtained and designated N and O (see figure 1). These two peaks were identified as GTP and UTP as follows: a) spectrophotometric characteristics of guanine and uridine nucleotides, with absorption maxima for peak N at pH 1 $=$ λ 256 mμ and for peak O at pH 2 $=$ λ 260-62 mμ. b) identical positions as those of known standards in column (6) and paper chromatography (20). c) analysis after hydrolysis with 2 N HCl for 1 hr at 100° C, as purine base and pyrimidine nucleotide, separated by the method of Wyatt (24), and d) enzymatic identification for peak O (UTP) by the nucleoside diphosphokinase reaction (3, 10, 13).

The molecular extinction coefficients taken into consideration were: for GTP at pH 1 and at λ 256 mμ $=$ 12.4 $\times$ 10^3 and for UTP at pH 7 and at λ 262 mμ $=$ 10 $\times$ 10^3.

In table II a summary of the qualitative and quantitative findings from five normal and two leukemic blood samples are presented.

Discussion

From the results obtained with normal and granulocytic leukemic blood, there appear to be no qualitative differences in the nucleotide pattern in these two types of blood. From the quantitative point of view, however, there are variations in the nucleotide content not only in normal but in leukemic blood. It is necessary to control the amount of hemoglobin and number of leukocytes present in the samples under study.

It will be noticed that in leukemic blood, in spite of having a decreased hemoglobin content and hematocrit volume, along with an increased number of leucocytes (see table I), the content of GTP and possibly other nucleotides are altered (see table II).

Among the nucleotides identified in this work, UDPAG and UTP have not previously been reported as being present in either type of blood sample. This may probably be due to the fact that the amount present in normal blood is very small (see table II). In this case it was possible to identify the UDPAG by the enzymatic technique, utilising the uridyl transferase from rat liver nuclei (13), and following the reaction spectrophotometrically as has been indicated before.

Previous workers have not found UTP in normal blood and it is quite possible that in such work, the peak

corresponding to GTP is a mixture of both GTP and UTP, since in our work done by column chromatography utilising Dowex 1 Cl′ and with HCl-NaCl gradient elution, it has been possible in every case to obtain two peaks which correspond to GTP and UTP.

The UTP was identified by the enzymatic method utilising the nucleoside diphosphokinase from pigeon breast muscle ([10]) and the spectrophotometric technique of Kornberg ([9]).

Summary

It has been possible to separate by column and paper chromatography from normal and leukemic blood the following nucleotides: DPN, TPN, AMP, ADP, ATP, UDPAG, UDPG, GTP and UTP. Some of them have been identified by enzymatic techniques, utilising the spectrophotometric methods of Kornberg. The only quantitative difference found, was an increased amount of GTP in leukemic blood. There were no differences in the contents of other nucleotides.

Acknowledgements

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FORMAS DE CONJUGACIÓN DE LOS CORTICOSTEROIDES URINARIOS

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Es bien conocido que la mayoría de los esteroides son eliminados en la orina del organismo humano, al estado conjugado y en un porcentaje relativamente pequeño, al estado libre.

La forma predominante de conjugación es con el ácido glucurónico. En 1936 Cohen y Marrian (1) aislaron el glucuronósido de estriol, el mismo año Venning y Brown obtuvieron el glucuronósido de pregnandiol (2) y sucesivamente los glucuronósidos de 3 α, 17 α, 20 α, trihidroxi-pregnane, 3 α, hidroxi-pregnane 20-one y últimamente los glucuronósidos de 3 α, 17 α, 21 trihidroxi-pregnane 11-20 dione (tetrahidrocortisona) y de 3 α, 17 α, 20 β, 21 tetrahidroxi-pregnane-11 ona (β cortolona) (3).

Otra de las formas de conjugación es con el ácido sulfúrico y han sido aislados los ésteres —sulfatos de la estrona (4), androsterona (5), dehidroepiandrosterona (6), etc.

También han sido halladas formas de conjugación de esteroides con aminoácidos (7).

El motivo del presente trabajo es mostrar los resultados obtenidos sobre la forma de conjugación de los corticosteroides eliminados en la orina humana luego de la acción estimulante de la hormona corticotrófica.

En los trabajos presentados en la literatura sobre conjugación de corticosteroides se describe la calidad de conjugación después de hidrólisis directa; en el siguiente estudio, por el contrario, se separa de los extractos urinarios tres fracciones: corticosteroides libres, sulfoconjugados y glucuronósidos; estas dos últimas fracciones son sometidas a hidrólisis específicas.

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MATERIAL Y MÉTODOS

1) *Material biológico.* — Se utilizaron extractos urinarios obtenidos de mezclas de orinas humanas luego de la administración de 80 U. I. de hormona adrenocorticotrófica-lenta, por vía intramuscular.

2) *Extracción y fraccionamiento.* — La extracción en la orina ha sido realizada por la mezcla éter/alcohol 3/1 v/v, adicionada con 50 % de sulfato de amonio (8).

Los extractos urinarios se disuelven en agua a pH 7; los corticosteroides

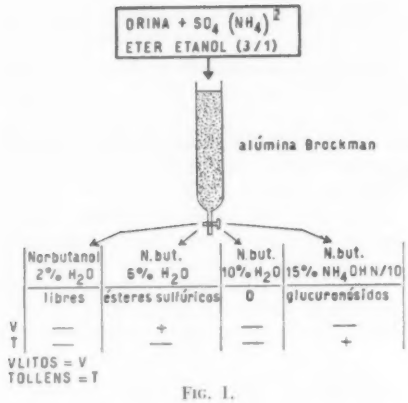

FIG. 1.

al estado libre son extraídos por el cloroformo; el resto se disuelve en butanol normal y cromatografiado sobre alúmina Brockmann, tipo II-III según el método de O. Crepy y M. Jayle (9) como lo muestra la Fig. 1.

La distribución del fraccionamiento cromatográfico sobre alúmina, de los ester-sulfatos y de los glucuronósidos está indicada en la Fig. 2.

La Fig. 2 muestra la presencia de dos porciones de ester-sulfatos, la primera completamente separada de los glucuronósidos y eliminada en las eluciones 2-6 % agua/butanol y la segunda fracción, que es eliminada por la elución 15 % NH_4OH N/10 en butanol junto con la primera parte de los glucuronósidos, ha sido separada en una segunda cromatografía sobre alúmina, cuyo interés se indica más abajo.

3) *Hidrólisis de esteroides conjugados.* — La fracción de ester-sulfatos ha sido hidrolizada por acción de la sulfatasa purificada y obtenida del jugo digestivo de la *Helix Pomacia* (12); los ester-sulfatos no hidrolizados por la enzima, lo fueron por acción de la mezcla dioxano-ácido tricloracético (13).

La fracción glucuronósida fué hidrolizada por la β glucuronidasa purificada (14).

4) *Extracción y purificación de los esteroides liberados.* — Los esteroides liberados por la hidrólisis fueron extraídos por el diclorometano, lavados con carbonato de sodio al 9 % 1/20 v/v y

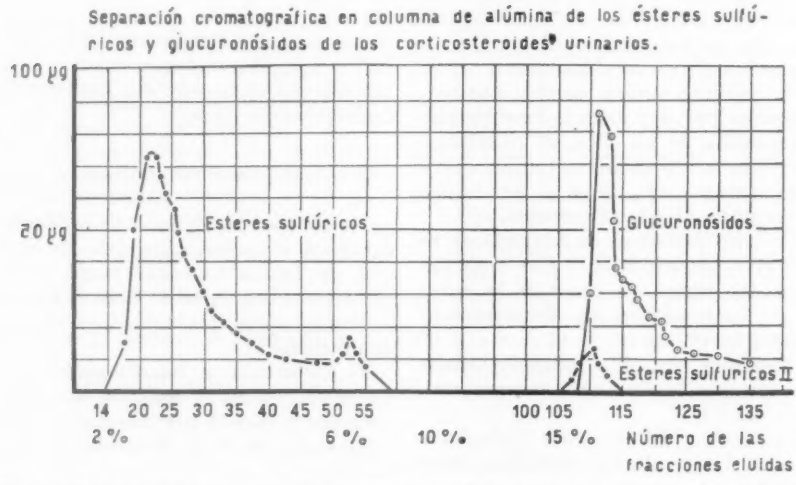
Separación cromatográfica en columna de alúmina de los ésteres sulfúricos y glucuronósidos de los corticosteroides urinarios.
100 µg
20 µg
Esteres sulfúricos
Glucuronósidos
Esteres sulfuricos II
14 20 25 30 35 40 45 50 55
100 105 115 125 135
2 %
6 %
10 %
15 %
Número de las fracciones eluidas

FIG. 2. — *Cada fracción eluida es valorada: los ester-sulfatos según la reacción del azul de metileno* (10), *y los glucuronósidos, utilizando la reacción de la naftoresorcina, de Tollens* (11).

ESQUEMA DEL FRACCIONAMIENTO CROMATOGRAFICO DE LOS CORTICOSTEROIDES

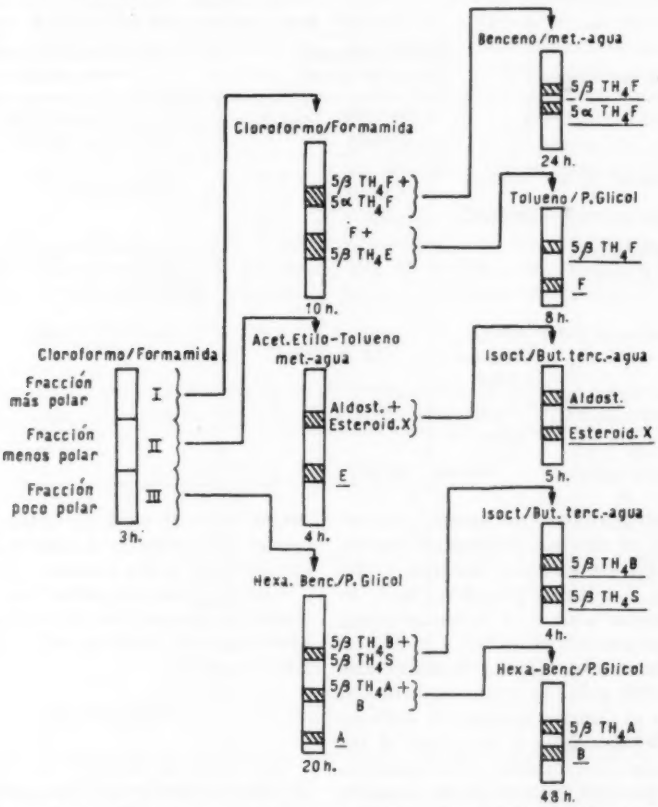

FIG. 3. — Nomenclatura: F: Δ_4*pregneno 11β,17α,21 triol-3,20 dione (hidrocortisona);* E: Δ_4*pregneno 17α,21diol-3,11,20 triona(cortisona);* B: Δ_4*pregneno 11β,21 diol-3,20 diona(corticosterona);* A:Δ_4*pregneno 21 ol-3,11,20 trione;* Aldost: Δ_4*pregneno 11β,21 diol-18al-3,20 dione;* 5βTH_4F: *5βpregnane 3α,11β,17α,21 tetrol-20 ona;* 5αTH_4F: *5αpregnane 3α,11β,17α,21 tetrol-20 ona;* 5βTH_4E: *5βpregnane 3α,17α21 triol-11,20 diona;* 5βTH_4B: *5βpregnane 3α,11β,21 triol-20 ona;* 5βTH_4A; *5βpregnane 3α21 diol-11,20 diona;* 5βTH_4S: *5βpregnane 3α,17α,21 triol-20 ona;* 5αTH_4B: *5αpregnane 3α11β21 triol-20 ona.*

dos veces con agua 1/20 v/v y purificados por cromatografía sobre florisil activado.

5) *Cromatografía sobre papel de los corticosteroides.* — Para aislar las hormonas córticosuprarrenales y algunos de sus metabolitos en las tres fracciones separadas (corticosteroides libres, sulfoconjugados y glucuronósidos), se utilizó el fraccionamiento cromatográfico sobre papel como lo indica la Fig. 3.

La mezcla de los corticosteroides se cromatografía primeramente en el sistema cloroformo/formamida en el que se separan tres fracciones según su polaridad: I) La fracción más polar

TABLA I

Identificación de los acetatos de corticosteroides aislados por cromatografía en papel

Naturaleza del acetato	Sistema cromatográfico					
	Metil-ciclohexano/ propilene glicol			Ciclohexano-benceno/ formamida		
		Esteroide aislado	Testigo		Esteroide aislado	Testigo
21-monoacetato de B	R_tDOC	0,19	0,19	R_f	0,30	0,30
21-monoacetato de A	R_tDOC	0,45	0,45		—	—
Acetato esteroide X $\Delta_4 - 3\,C = O$				R_t18-21 diacetato aldosterona	0,47	1
3-21-diacetato de TH_4F	R_t3-21-diacetato TH_4E	0,61	0,63	R_f	0,48	0,47
3-21-diacetato de TH_4E	R_t3-21-diacetato TH_4E	1	1	R_f	0,65	0,63

se cromatografía sucesivamente: nuevamente en el sistema cloroformo/formamida y en los sistemas benceno/metanol-agua y tolueno/propilen-glicol, lo cual permite aislar: la hidrocortisona, 5 α y 5 β tetrahidrocortisol y la 5 β tetrahidrocortisona. II) La fracción medianamente polar se cromatografía primero en el sistema acetato de etilo-tolueno/metanol-agua y luego en el sistema isooctano/butanol terciario-agua, lo cual permite separar la cortisona, la aldosterona y un esteroide X. III) La fracción menos polar es cromatografiada primero en el sistema hexano-benceno/propilen-glicol y en los sistemas isooctano/butanol terciario-agua y hexano-benceno/propilen-glicol para aislar: la corticosterona, la 11-dehidrocorticosterona, la 5 β tetrahidrocorticosterona, la 5 β tetrahidro-11-dehidrocorticosterona, la 5 β tetrahidro-17-hidroxi-11-desoxicorticosterona y la 5 α tetrahidro-corticosterona.

La identificación de cada corticosteroide ha sido realizada por la absorción en el ultravioleta a 254 mμ, por la reacción con el azul de tetrazolium, y por la fluorescencia al hidróxido de sodio 2N y al ácido fosfórico 15 %.

Ciertos corticosteroides han sido acetilados y los acetatos identificados por cromatografía sobre papel, según lo indica la tabla I.

Resultados

La tabla II muestra la repartición de las hormonas córticosuprarrenales

TABLA II

Hormonas corticosuprarrenales urinarias (después de 80 U.I. de ACTH lenta)

	I Libres µg ‰	II Sulfoconjugadas µg ‰	III Glucurononósidos µg ‰
F	300	200	260
E	15	20	60
B	trazas	240	0
A	trazas	70	0
Total µg ‰	*315*	*530*	*320*
% Total General (I + II + III)	26 %	46 %	28 %

en las fracciones libre, sulfoconjugada y glucuronósida. Los resultados han sido expresados en $\mu g/L$ de orina. Estos resultados han sido obtenidos como promedio de dosajes cuantitativos hechos según las reacciones: del azul de tetrazolium, de la fenilhidrazina y de la medida del formol obtenido por oxidación con ácido peryódico.

Discusión

Luego de la estimulación por la hormona adrenocorticotrófica se encuentra en las fracciones libres principalmente hidrocortisona; esta hormona se halla repartida también en las fracciones sulfoconjugada y glucuronósida. La cortisona se encuentra en ambas fracciones.

Por el contrario la corticosterona y la 11-dehidrocorticosterona se encuentran exclusivamente en la fracción sulfoconjugada. El mismo resultado ha sido obtenido anteriormente en una mezcla de orina estudiada en iguales condiciones (15). Esta conclusión resulta interesante por el hecho bien conocido que el "clearance" de las hormonas libres, sulfoconjugadas y glucuronósidas es diferente.

La totalidad de la corticosterona y de la 11-dehidrocorticosterona han sido liberadas por la sulfatasa. La actividad de dicha sulfatasa se ha determinado utilizando como substracto la Cortisona-21-Sulfato. Este hecho sugiriría la conjugación de la corticosterona en C_{21}. Otra de las pruebas de tal posible conjugación es la obtención por síntesis biológica del sulfato de corticosterona (16).

Como conclusión de los resultados presentados en el cuadro II se observa que el mayor porcentaje de las hormonas corticosteroides activas son eliminadas en la fracción sulfoconjugada.

El cuadro III representa los valores obtenidos de los metabolitos tetrahidrogenados de los corticosteroides.

Como se observa la repartición de los metabolitos tetrahidrogenados es completamente diferente de la de los corticosteroides activos. La mayor parte de estos esteroides se encuentran en la fracción glucuronoconjugada (aproximadamente 90 %), el resto se reparte en las otras fracciones.

El principal metabolito corticosteroide es la tetrahidrocorticosterona (5 β TH_4F); merece subrayarse que la relación TH_4E/TH_4F que según diferen-

TABLA III

Metabolitos tetrahidrogenados de las hormonas corticosuprarrenales urinarias
(después de 80 U. I. de ACTH-lenta)

	I Libres μg ‰	II Sulfoconjugadas μg ‰	III Glucuronósidos μg ‰
5 β TH_4F	300	125	7500
5 α TH_4F	0	0	300
5 β TH_4E	55	0	1300
5 β TH_4S	trazas	trazas	450
5 β TH_4B	100	100	800
5 α TH_4B	0	0	3000
5 β TH_4A	trazas	260	450
Total μg ‰	455	485	13800
% Total General I + II + III	3 %	3,5 %	93,5 %

tes autores es superior a 1, en el presente trabajo resulta del orden de 0,2.

Los metabolitos de la corticosterona constituyen aproximadamente el 30 % de los metabolitos estudiados, siendo el principal de ellos la 5 α tetrahidro-corticosterona.

La segunda fracción éster-sulfato, mencionada antes, ha sido hidrolizada por la sulfatasa y el estudio cromatográfico sobre papel es presentado en la Fig. 4. La mayor parte de esta fracción lo constituye un esteroide cuyas características son muy próximas de la TH_4DOC. El hecho que dicha fracción sea fuertemente polar y que la relación cuantitativa Éster-Sulfato/esteroide sea mucho mayor que en la primera fracción sulfato, hace pensar en otra forma de conjugación.

Estudiando la conjugación de la aldosterona fué sorprendente encontrar valores varias veces superiores a las cifras normales obtenidas en la literatura, utilizando el método de Neher y Wettstein (17). Este hecho sugirió profundizar dicha anomalía y utilizando tres sistemas sucesivos de cromatografía sobre papel según lo indica la Fig. 5, se obtuvo un nuevo esteroide. Dicha figura representa primero la cromatografía en el sistema cloroformo/formamida; luego la región de la cortisona es cromatografiada en el sistema acetato de etilo-tolueno / metanol-agua (Bush C), la zona de la aldosterona es

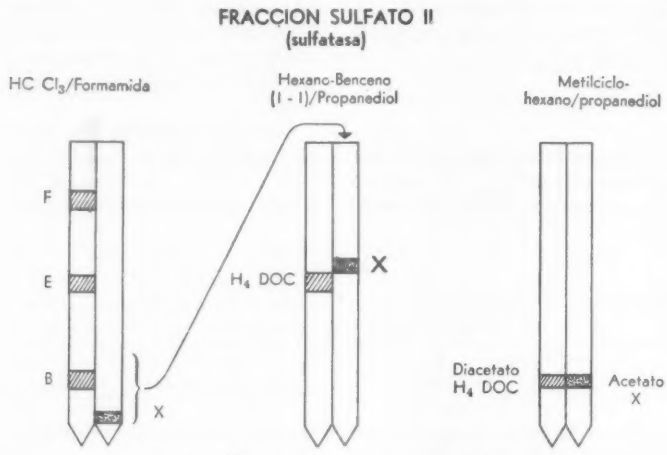

FIG. 4. — H_4DOC = *5βpregnane 3α.21 diol-20ona.*

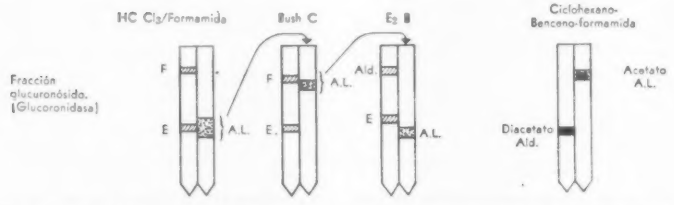

FIG. 5. — F: *hidrocortisona;* E: *cortisona;* Ald: *aldosterona;* A. L.: *Esteroide X.*

nuevamente cromatografiada en el sistema isooctano/butanol terciario-agua, lo cual permitió separar el nuevo esteroide X de la aldosterona, siendo su R_t con respecto a esta última de 1,50. El R_t del acetato de dicho esteroide X es con respecto al 18-21 diacetato de aldosterona de: 0,47 en el sistema ciclohexano-benceno/formamida.

Las características físico-químicas de dicho esteroide son las siguientes:

Reacción al azul de tetrazolium: débilmente positiva.

Absorción a 240 $m\mu$: positiva.

Fluorescencia al HONa en la lámpara de Wood: positiva.

Fluorescencia al ácido fosfórico: negativa.

Máximo de absorción en el espectro ultravioleta: 238,5 μm.

Máximo de absorción en el espectro al ácido sulfúrico' 278 μm.

Conclusiones

Las principales conclusiones de estas investigaciones son las siguientes:

1) La mayor parte de los corticosteroides activos (cortisona, hidrocortisona, corticosterona y 11-dehidrocorticosterona) luego de la administración de adrenocórticotrofina son eliminadas por la orina, especialmente en la fracción sulfoconjugada.

2) La totalidad de la corticosterona y de la 11-dehidrocorticosterona han sido encontradas exclusivamente en la fracción éster-sulfato.

3) La mayor parte de los metabolitos tetrahidrogenados de los corticosteroides son glucuronósidos.

4) La relación TH_4E/TH_4F desciende intensamente luego de la administración de la adrenocórticotrofina.

5) El principal de los metabolitos de la corticosterona lo constituye el 5 α tetrahidrocorticosterona.

6) Un esteroide $\triangle_4$ 3 C = O ha sido aislado en la fracción glucuronosida.

7) La separación de una segunda fracción sulfoconjugada, conteniendo un esteroide muy próximo de la tetrahidro-11-desoxicorticosterona.

Resumen

Los corticosteroides fueron separados en tres fracciones: libres, sulfoconjugados y glucuronósidos. La corticosterona y la 11-dehidrocorticosterona han sido encontrados exclusivamente en la fracción sulfoconjugada. Los metabolitos tetrahidrogenados del cortisol y de corticosterona son en su mayor parte glucuronósidos. Un nuevo esteroide $\triangle_4$ 3 C = O ha sido encontrado en la fracción glucuronósida.

Resumé

Les cortico-stéroïdes urinaires ont été séparés en trois fractions: libre, sulfoconjugués et glucuronosides. La corticosterone et la 11-dehydrocorticosterone ont été trouvées exclusivement dans la fraction sulfoconjugée. Les métabolites tetrahydrogenés du cortisol et de la corticosterone sont en majeure partie glucuroconjugués. Un nouveau steroïde $\triangle_4$ 3 C = O a été trouvé dans la fraction glucuronoside.

Summary

The urinary corticosteroides were separated into three fractions: free, sulphates and glucuronosides. The corticosterone and the 11-dehydrocorticosterone were found exclusively in the sulphate fraction. The tetrahydrogenated metabolites of cortisol and corticosterone are mainly in the glucuronoside fraction. A new steroid $\triangle_4$ 3 C = O was found in the glucuronoside fraction.

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INFLUENCE OF THE GONADS ON BLOOD CLOTTING FACTORS (*)

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CLOTTING of the blood is one of the several mechanisms that may be involved in atherosclerotic occlutions. Sexual differences in the distribution of clinical atherosclerosis in the human being may indicate that gonadal factors could bear some influences in blood clotting mechanisms. In monkeys, changes in fibrinogen levels have been reported in connection with the menstrual cycle (1). Gillman and Naidoo (2) demonstrated in rats differences in fibrinogen levels and in spontaneous fibrinolytic activity in connection with sexual factors; we have extended these studies and partly confirmed such findings.

MATERIAL AND METHODS

White albino rats of mixed breed, weighing from 150 to 300 grams on a standard laboratory diet, were used. The animals were separated into severals groups: 1) intact males, 2) castrated males, 3) intact females, and 4) spayed females. Since small changes in the fibrinogen content is seen in different estrous phases of females rats (unpublished) and in order to avoid these possible variations, in group 3, 12 rats were equally divided into four different estrous phases.

The effects of the administration of hormones were determined in the following eight groups of gonadectomized animals: 5) control males, 6) males injected with testosterone, or 7) with estradiol, 8) control females, 9) females injected with estradiol, 10) with progesterone, 11) with estradiol and progesterone, and 12) with testosterone (***). Each group, unless otherwise stated, consisted of 15 animals.

Gonadectomy was performed under intraperitoneal pentobarbital anesthesia (40 mg/Kg of a 0,4 % solution) using a clean but not sterile technique

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(***) Estradiol. Δ 1, 3, 5 (10), estratriene, 3, 17 βol; progesterone: Δ 4, pregnene, 3, 20 one; testosterone: Δ 4, androstene, 3, one, 17 βol; were kindly supplied by Dr. Esteban Montuori from Laboratorios Dr. Gador, Buenos Aires.

and confirmed in females by continuous diestrus. Hormones as microcrystals suspended in an aqueous vehicle were given as follows: groups 6 and 12 received intramuscular injections of 100 μg of testosterone; groups 7 and 9 were injected with 25 μg of estradiol; group 10 was injected with 1 mg of progesterone; groups 5 and 8 were injected with vehicle. All the animals received three injections separated by three days intervals and were sacrificed 24 hours after the last injection. Group 11 received 25 μg of estradiol and 1 mg of progesterone 4 days apart; sacrifice was performed 96 hours later. Vaginal smears were performed daily and interpreted freshly under the microscope; animals were sacrificed forty days or more after gonadectomy.

All rats were anesthetised as above and, following laparotomy, blood was withdrawn from the aorta into 3,8 % sodium citrate making a final concentration of 9:1, hemolysis was carefully avoided and the hematocrit value determined at 3000 rpm during 30 minutes. Fibrinogen was determined as fibrin with a method slightly modified from Ratnoff and Menzie ([3]) and Bidwell ([4]), as modified by Biggs and Macfarlane ([5]). Blood was centrifuged at 1500 rpm during 15 minutes and the plasma separated. Then, 0,2 ml of this plasma was diluted into 4,8 ml of saline-veronal-acetate buffer solution at pH 7,4. It was clotted with 0,2 ml of 0,1 M calcium chloride and 0,2 ml of a thrombin solution (Cruostyl, 1000 Mellamby units/100 ml) and then allowed to stand 15 minutes at 37° C. The clot was centrifuged with eight glass beads, carefully washed three times with cold saline solution, hydrolyzed with 2 ml of 0,1 N NaOH at 100° C during 10 minutes and finally acidified with 0,1 ml of 7 N ClH. The tyrosine content was determined with a Hilger spectrophotometer at 275 mμ against a blank containing 2 ml of 0,1 N NaOH and 0,1 ml of 7 N ClH. To determine spontaneous fibrinolytic activity, the same procedure was repeated after incubating the clot at 37° C during 4 hours. Plasma fibrinogen levels expressed in μg of tyrosine per ml were corrected for citrate dilution and hematocrit values according with Guest et al. ([6]).

All determinations were performed by triplicate; error of the method was found to be $\pm$ 2,5 % in 18 determinations grouped by triplicates and performed in the same plasma sample. Results were compared with Student's "t" test accordingly with Fisher's tables ([7]); values differing by more than 3 standard deviations from the mean, were deleted.

Results

Table I shows that males have higher fibrinogen levels than females and that these differences disappear after gonadectomy. Fibrinolysis was similar in both sexes and not affected by castration in the male, although it was reduced in spayed females.

Table II shows that testosterone increases fibrinogen levels in castrated males without affecting fibrinolysis, while estradiol was without effect. In spayed females, no changes were produced by estradiol while progesterone and testosterone increased fibrinogen levels; fibrinolysis was enhanced by the administration of progesterone.

Discussion

Our results confirm in the main, the conclussions reported by Gillman and Naidoo ([2]), i. e., that male rats have higher fibrinogen levels than females and that these differences disapear after castration, since gonadectomy depresses plasma fibrinogen levels only in males. In connection with fibrinolysis we could not totally du-

TABLE I

The effect of castration on plasma fibrinogen and fibrinolytic activity in rats

Number	Group (*)	Fibrinogen level (**)		Fibrinolytic activity (**)	
		Number of rats	Tyrosine μg/ml	Number of rats	% Fibrin clot lysis
1	Intact males	11	833,83 ± 91,36	7	21,57 ± 5,02
2	Castr. males	10	594,57 ± 45,54	10	27,08 ± 5,98
3	Intact females	11	476,14 ± 30,45	12	30,57 ± 3,75
4	Spayed females	11	512,33 ± 19,48	7	8,72 ± 3

Group	Fibrinogen level		Fibrinolytic activity	
	"t"	p	"t"	p
1 vs 2	4,430	< 0,001	0,705	> 0,40
1 vs 3	8,510	< 0,001	1,440	> 0,10
3 vs 4	1,000	< 0,30	4,370	< 0,001
2 vs 4	1,661	< 0,10	2,744	< 0,02

(*) Castr., castrated.
(**) Mean ± standard error.

TABLE II

The effect of hormones on plasma fibrinogen and fibrinolytic activity in gonadectomized rats

Number	Group (*)	Fibrinogen level (**)		Fibrinolytic activity (**)
		Number of rats	Tyrosine μg/ml	% Fibrin clot lysis
5	Control castr. males	8	598,38 ± 57,98	8,62 ± 2,79
6	Castr. males + testosterone	7	738,92 ± 25,47	4,48 ± 2,19
7	Castr. males + estradiol ..	10	505,53 ± 21,44	10,74 ± 2,91
8	Control spayed females ..	11	564,35 ± 35,28	7,95 ± 2,76
9	Spayed + estradiol	10	600,68 ± 41,11	8,99 ± 2,34
10	Spayed + progesterone ..	8	690,64 ± 33,62	22,37 ± 6,34
11	Spayed + estrad. + progesterone	6	629,65 ± 73,48	30,41 ± 7,76
12	Spayed + testosterone ...	9	692,74 ± 10,97	5,85 ± 2,8

Group	Fibrinogen level		Fibrinolytic activity	
	"t"	p	"t"	p
5 vs 6	2,217	< 0.05	1,175	> 0.20
5 vs 7	1,502	> 0.10	0,519	> 0.60
8 vs 9	0,670	> 0.50	0,287	> 0.70
8 vs 10	2,593	< 0.05	2,063	> 0.05
8 vs 11	0,777	> 0.40	2,726	< 0.02
8 vs 12	3,479	< 0.01	0,534	> 0.60

(*) Castr., castrated; estrad., estradiol-17 β.
(**) Mean ± standard error.

plicate their results: the amount of lysis has been consistently lower in our rats and although normal animals had similar values in both sexes, castration depressed fibrinolysis only in females; these differences cannot be accounted for at the present.

Our observation that testosterone raises plasma fibrinogen levels in gonadectomized animals, were also in accordance to those reported by Gillman et al. ([8]). A similar effect was seen by us after progesterone administration. These results as well as those connected with changes in fibrinolytic activity brought about by gonadectomy in females, and by the administration of progesterone in spayed rats, further confirm the role of the gonads in controlling blood factors involved in clotting.

Other endocrine glands may also play some part in this connection; for instance, the hypophysis through ACTH liberation and the adrenals through corticoid secretion, are apparently able to influence blood fibrinogen levels ([9]), as also attested by sedimentation rate changes seen in patients after the administration of these hormones ([10, 11]). Furthermore, Campbell et al. demonstrated that plasma fibrinogen levels are raised in dogs when diabetes is produced with purified growth hormone ([12]). The possible endocrine control of other blood clotting factors, besides fibrinogen, is also worth of consideration. Connection of these mechanisms to the problem of atherosclerosis must await further exploration.

Summary

Plasma fibrinogen, expressed as tyrosine liberated from precipitated fibrin, and spontaneous "in vitro" fibrinolysis, were determined in rats. A clear sexual difference in plasma fibrinogen levels, abolished by castration was demonstrated. Testosterone and progesterone were able to elevate the plasma fibrinogen levels reduced by gonadectomy; estradiol was without any effect. Oöphorectomy in females reduced fibrinolysis, while administration of progesterone enhanced clot lysis. The possibility that different endocrine glands may control factors involved in blood clotting mechanisms was discussed.

Acknowledgements

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We wish to express our appreciation to Dr. Jaime A Moguilevsky who studied some of the smears and to Enrique A. Savino for technical assistance.

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PROCEEDINGS OF THE SOCIETY OF BIOLOGY OF RIBEIRAO PRETO

(SÃO PAULO, BRASIL)

June 13, 1959

The growth of hair in hemidecorticate rats. MIGUEL R. COVIAN AND JOSÉ ANTUNES R. *(Departamento de Fisiologia, Faculdade de Medicina, Ribeirão Preto, Sao Paulo, Brasil).*

Male and female hemidecorticate rats show a delay in hair growth.

Archicortex is the part of cerebral cortex responsible for this result.

In hemiaarchidecorticate-castrated rats, hair grows much quicker than in hemiarchidecorticate rats.

It is advanced that the slowing of the hair growth observed can be attributed to an interference with the role played by the different hormones that regulate the pilory system being the gonads the main factor.

Junio 13, 1959

Studies on techniques using single-solution stains for intestinal protozoa. M. PEREIRA BARRETTO AND H. ZAGO FILHO. *(Departamento de Parasitologia, Faculdade de Medicina, Ribeirão Preto, Sao Paulo, Brasil).*

A careful evaluation of the relative merits of the following single-solution staining techniques recommended for intestinal protozoa was made: Mayer's acid hemalum, Weigert's iron-hematoxylin, Walker's iron-hematoxylin, Mayer's hemalum modified by Lillie, Ratcliffe and Parkins' phosphotungstic acid-hematoxylin, Noble's iron hematoxylin, Goldman's iron-hematoxylin and Lawless' acid fuchsin-fast green stain.

Only Mayer's hemalum and Lillie's modification proved satisfactory, meeting the following requirements: stability of solutions for a long period of time, consistency of results, regularity of staining trophozoites and cysts (particularly the cysts of *Entamoeba coli* which are difficult to stain), good density and contrast of color, no need for unstaining (differentiation) and rapidity. Although morphological details are not as well brought out as in some other two-solutions staining methods, they are clear enough to allow the differential diagnosis of the various species of protozoa quite easy.

Mayer's hemalum is better, so far as morphology is concerned, but is time consuming, since cysts of amoebae take at least 15 minutes to stain. Lillie's technique is slightly inferior, but it is time saving and not expensive good staining of cysts being obtained in less than 5 minutes.

November 11, 1959

A simplified method for the study of chromosomes in man. CÁSSIO BOTTURA AND IRIS FERRARI. *(Departamento de Clínica Médica, Faculdade de Medicina, Ribeirão Preto, Sao Paulo, Brasil).*

Great advances in cytogenetics were determined by the use of a technic after pretreatment

of cells with hypotonic solution and colchicine.

In man, culture of the tissue to be studied seems to be an obligatory step in the execution of the Feulgen "squash" technic (1-3).

Searching for a simplified technic, we tried to submit the cells "in vivo" to the action of a derivative of colchicine-desacetylmethylcolchicine, thus avoiding tissue culture.

Initially, the drug was used in patients with malignant diseases and afterwards in normal ones. After preliminary trials, the dose of 0.1 mg per kilogram of body weight, was diluted in saline and slowly administered intravenously. After two hours, bone marrow was obtained by sternal puncture, treated with hypotonic solution and stained with Feulgen reagent, according to the technic described by Ford *et al.* (4). A total of 8 individuals were studied in this way.

After squashing, the preparation showed a fair number of metaphases, with good spreading of chromosomes which disclosed the characteristic effects of the drug. Counting, sex determination and individual study of chromosomes could be easily carried out.

The simplicity of the method lead us to use it in three patients for the study of the chromosomes of malignant cells in ascitic and pleural fluids. The drug was injected in abdominal and pleural cavities and the results were the same as for bone marrow cells.

All individuals were followed clinically and hematologically and no abnormality was detected that could represent toxic effects of the drug. Moreover, the dose used is far less than the one utilized in therapy of neoplastic diseases or gout in man or in animal experiments.

We believe the same method will be useful for chromosomal studies in other human tissues, whether neoplastic or not.

Better adjustment of the dose and proper choice of the time elapsed between its administration and collection of cells will, doubtless, improve the technical qualities of the preparations.

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Lactate oxidation by cell-free extracts from *Propionibacterium pentosaceum*.

F. J. S. LARA. *(Departamento de Bioquímica, Faculdade de Medicina, Ribeirão Preto, Sao Paulo, Brasil).*

Re-investigating the nature of the lactic dehydrogenase occurring in *P. pentosaceum* it was found that heating the preparations at 50° C during 5 minutes reduced the activity as measured with 2:6-dichlorophenol, indophenol or phenazine methosulphate as electron acceptors, whereas that measured with ferricianide was essentially unaltered.

Attempts of purification of the enzyme based on the ferricianide assay revealed that reduction of this substance in the presence of lactate, by these preparations, requires at least two fractions, one of which is stable to heating at 100° C during 5 minutes. This heat stable fraction is not dyalisable and is precipitated by 50 % saturation with ammonium sulphate.

PROCEEDINGS OF THE ARGENTINE SOCIETY OF BIOLOGY

(CÓRDOBA, ARGENTINA)
June 11, 1959

Excretion of total neutral 17-ketosteroids in normal adults. A. CANDIOTTI, A. MARTIN AND E. NOWOTNY. *(Cátedra de Química Biológica, 2ª parte, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Argentina).*

The authors studied the excretion of the total neutral urinary 17-ketosteroids from 100 normal adults, 20 to 40 years of age using the Bercovich and col. method. The following results were found: for males, the excretion ranged from 6.58 to 18.33 mg in 24 hours, with an average of 11.95 ± 3.23. The values for the females ranged from 4.10 to 14.41 mg in 24 hours, with an average of 8.24 ± 2.31.

Since the physiological variations can be included between two standard deviations, we could accept as normal values for excretion in the males 5.49 to 18.41 mg in 24 hours, and for females 3.62 to 12.86 mg in 24 hours.

We confront these results with those obtained from other authors which have used similar or different methods, and the results are discussed.

Titration of total neutral 17-ketosteroids in urine. A. V. BERCOVICH, A. CANDIOTTI, A. MARTIN AND E. NOWOTNY. *(Cátedra de Química Biológica, 2ª parte, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Argentina).*

The authors suggest new modifications of the method of Drekter, Pearson, Bartckzak and Mc Gavack for the evaluation of total neutral 17-ketosteroids from urine and of the modifications introduced by Pearson and Giaccione, and by Marenzi and Braegger. Their purpose is to make the method more versatile, avoiding the use of aliquots from hydrolysed material and to facilitate the reading of the stained reaction, by raising the volume of the solution.

Some technical factors were examined, such as, concentration of potassium hydroxide, incubation time, interference from pigments and chromogens in the stained reaction, and fulfillment of the Beers' law.

Duplicate determinations and recoveries were performed.

Glucose release in the isolated liver of "Bufo arenarum Hensel". I. FLORES. *(Instituto de Fisiologia "O. Orias", Facultad de Ciencias Médicas, Santa Rosa 1085, Córdoba, Argentina).*

The liver of 288 toads *Bufo arenarum* Hensel, weighing 50 to 225 g was perfused, all the year long. Ringer solution was used, for periods of even 9 hours, at a temperature of 26° to 32° C and a pressure of 45 cm of water.

It was possible to observe: a) increasing the perfusion volume, increased the quantity of liberated glucose per gram of body or liver, maintaining an arithmetical progression; b) in-

creasing the perfusion volume per unit of weight of animal or liver, the concentration of glucose decreased to attain a minimum level; c) in prolonged perfusions, 9 hours long, it was observed a slight increase that reached to a ten per cent of the initial value.

Glycemia in "Bufo paracnemis" and glucose release by the liver. N. I. PERASSI. *(Instituto de Fisiología "O. Orías", Facultad de Ciencias Médicas, Santa Rosa 1085, Córdoba, Argentina).*

The isolated liver of 18 *Bufo paracnemis*, weighing 260 to 850 gr was perfused.

It was possible to observe a fall in the glycemia, as the animals were kept enclosed and unfed during several months (from February to July). Glycemia in these animals is higher than in *Bufo arenarum* (52.6 mg/100 ml in *Bufo paracnemis* and 29.4 mg/100 ml in *Bufo arenarum*). The ponderal relation between liver and body is less in *Bufo paracnemis* than in *Bufo arenarum*; it leads to the assumption that the liver is proportionally bigger as smaller is the animal. There is a direct relationship between perfusion volume and quantity of liberated glucose per unit of time and per gram of liver or body weight. Taking into account the perfusion rate, there is a greater liberation of glucose by liver in *Bufo paracnemis* than in *Bufo arenarum*; glycemia and the quantity of glucose liberated are directly related. During the perfusion, glucose liberation was constant.

(BUENOS AIRES, ARGENTINA)
September 3, 1959

Effect of hydrocortisone and desoxycorticosterone in partially nephrectomized rats drinking NaCl and KCl solutions. J. C. FASCIOLO, M. CABUT AND A. JIMENA. *(Departamento de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina).*

Normal rats and those with 60-70 % reduction of the renal tissue were placed in metabolic cages, and the liquid consumed as well as the diuresis and Cl excretion were measured, when drinking water and when the water was replaced by solutions of NaCl and KCl.

Both the normal and the partially nephrectomized group showed an increase in the volume drunk and in diuresis when water was replaced by a 1 % NaCl solution. The 1 % KCl solution slightly reduced the volume drunk by the normal rats, but caused a drastic reduction in the ingestion and the diuresis of the partially nephrectomized group. Hydrocortisone, 2 mg per rat daily during ten days, caused a small increase in the volume drunk by the normal rat drinking 1 or 2 % NaCl solution; on the contrary, a tendency towards reduction of ingestion and diuresis was observed in the partially nephrectomized rats drinking 1 % NaCl solution. The changes produced by hydrocortisone in normal rats drinking 1 or 2 % KCl solution were small; nevertheless a reduction in the ingestion of 1 % KCl was found in the rats with reduced kidneys. Desoxycorticosterone acetate (2 mg/rat during 10 days) increased the volume of 1 and 2 % NaCl solution drunk by the normal rats, but caused a slight reduction in the partially nephrectomized group drinking 1 % NaCl. Desoxycorticosterone acetate increased slightly the volume of the 1 % KCl solution drunk by the rats with renal mass reduced, and did not change significantly the volume drunk by the normal ones.

Neurosecretory material in ganglionary cells of the trigeminus in the rat. E. M. RODRÍGUEZ. *(Instituto de Histología y Embriología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza).*

The histological study of the rat's fifth pair has shown:

1) Ganglionar cells scattered along the middle third of the principal sensitive branch. 2) These cells present in their cytoplasm a Gomori positive substance; and 3) The cytologic characteristics of these cells resemble those of neurosecretory cells.

Antigenic relationship between hemorrhagic fever virus in the province of Buenos Aires and spring-summer Russian encephalitis. A. S. PARODI, N. METTLER, M. BOXACA, L. B. DE GUERRERO, F. GARZÓN. *(Cátedra de Microbiología y Parasitología, Facultad de*

Ciencias Médicas, Paraguay 2155, Buenos Aires).

The hemorrhagic fever virus of the Argentine outbreak is neutralized "in vivo" by Russian encephalitis immuno serum in significative amounts.

This enables us to include the hemorrhagic fever virus in the B group of Arbor virus and with the European and Asian encaphalitis or hemorrhagic fever.

Some considerations about the clinical features produced by these virus are made.

Vaginal threshold to estradiol benzoate. R. NALLAR. *(Instituto de Biología y Medicina Experimental, Obligado 2490, Buenos Aires).*

Three vaginal reactions are produced by gradually increasing doses of estrogens injected daily to ovariectomized rats.

Suboestrus: With 0.001, 0.01 and 0.02 μg of estradiol benzoate, some cornified and basophilic cells are observed not reaching proestrus or oestrus.

Periodical oestrus: Daily doses of 0.03 and 0.04 μg produce oestrus and metaestrus.

Continued Oestrus: With 0.05 μg of estradiol benzoate, a continued oestrus is observed, becoming periodical 15 to 20 days after beginning administration.

Studies on toad's isolated tissue metabolism. Action of thyroid hormones. A. O. DONOSO. *(Departamento de Fisiología, Faculdade de Medicina de Ribeirão Preto, São Paulo, Brasil).*

The metabolism of toad's isolated tissues was studied with Warburg manometric technique.

1) The oxygen consumption of kidney and liver had a linear relationship with temperature between 10—30° C. In heart slices that relationship was observed between 10—25° C. At 30° C it showed alterations.

2) Captivity did not modify the metabolism.

3) Seasonal variations were found in the oxygen consumption of normal toads.

4) Previous treatment with L-thyroxine or L-Triiodothyronine (1 mg/day/4 days), produced at 25° C an increase in the metabolism of kidney and heart, but not in liver and muscle.

5) Thyroidectomized toads (with I^{131}), had no modifications in kidney, liver, heart, muscle, or skin respiration.

September 10, 1959

Histochemical study of postnatal development of skin connective tissue. R. E. MANCINI, O. VILAR, E. STEIN AND H. FIORINI. *(Instituto de Anatomía General y Embriología, Facultad de Ciencias Médicas, Paraguay 2155, Buenos Aires).*

A study was carried out in the morphological and histo and cytochemical modifications of the rat skin connective tissue from birth to maturity. It was observed: 1) progressive diminution of the interfibrilar mucopolysaccharides from birth to maturity; 2) progressive increase with age of the number and thickness of the collagen fibers with production of crossed collagen bundles in the adult age; 3) elastic fibers appeared at 12th. day and then thickened till the adult age; 4) basal membrane was thin in the early days and then its thickness increased with deeper stain with PAS and more intense silver impregnation; 5) early fibroblasts changed into typical adult connective tissue cells (fibrocytes) with gradual diminution of cytoplasmatic nucleoproteins and PAS positive material; 6) lacking of stricking morphological or cytochemical changes of the mast-cells in the different ages; 7) more intense enzymatic reactions in the mast-cells than in the fibroblasts.

Incorporation of S^{35}, adenine-C^{14} and methionine-S^{35} through the skin connective tissue during postnatal development. R. E. MANCINI, H. FIORINI AND E. STEIN. *(Instituto de Anatomía General y Embriología, Facultad de Ciencias Médicas, Paraguay 2155, Buenos Aires).*

A radioautographic study of the rat skin connective tissue was carried out at different ages using radioactive sulphur (S^{35}), methionine-S^{35} and adenine-C^{14}. The following observations were made: 1) The embryonic and early post-natal connective tissues trapped more S^{35} than the adult one. S^{35} was trapped first by fibroblasts and then by the intercellular ground-substance. Mast-cells incorporated move S^{35} than fibroblasts though there

were no striking differences at various ages as in the case of fibroblasts. 2) Using adenine-C^{14} and methionine S^{35}, localization of the radioisotope was seen more in both the fibroblasts and mast-cells than in the intercellular ground-substance, but in the fibroblasts more trapping was seen in the embryonic and early post-natal connective tissues than in the adult one. 3) The incubation of slides with barium hydroxide, hyaluronidase and perchloric acid, showed that in all cases S^{35} is incorporated by sulphated mucopolysaccharide, adenine-C^{14} is taken by the nucleoproteins and methionine-S^{35} by the proteins.

On the basis of the utilized radioisotopes we postulate the relation among the metabolic activity of the fibroblasts and the maturation and the differentiation of the connective tissue from birth to the adult age.

Amilasemia in the toad. E. LEVIN AND R. W. DE LEVIN. *(Instituto Nacional de Microbiología Malbrán, Ministerio de Salud Pública, Buenos Aires).*

1) High values for blood amylase in the toad *Bufo arenarum* Hensel are confirmed, with a mean of 8 500 Somogyi units, determined on 400 animals with a typical deviation of ± 6 000 for 66 % of the animals of the group. 2) Removal of the pancreas decreases by 51 % the blood amylase levels 48 hours after operation, in relation to normal controls. 3) Conversely, hepatectomy produces an increase of 101 % of the circulating enzyme 48 hours after operation. 4) Removal of the digestive tract has no influence on the amylase values 48 hours after operation.

Relationship between glycemia and blood amylase in the toad. E. LEVIN AND R. W. DE LEVIN. *(Instituto Nacional de Microbiología Malbrán, Ministerio de Salud Pública, Buenos Aires).*

1) An inverse relationship between glucose and blood amylase is observed. Under hypoglycemia, a hyperamylasemia is observed and, on the contrary, hyperglycemia provokes a fall in amylasemia. 2) This correspondence seems to be under para-sympathetic control; on sectioning the pneumogastric nerves, variations of glycemia do not modify the normal levels of blood amylase. 3) It is concluded that the pancreas has no direct role or a very reduced one, in the production of blood amylase in the toad. Pancreatectomy or hepatectomy followed by cutting of the pneumogastric nerves does not significantly modify the amylasemia. The amylase fall obtained by pancreatectomy should be attributed to the resulting hyperglycemia.

Antitetanic and antidiphtheria vaccine associated with protamine and other coadyuvant substances. F. MODERN. *(Instituto Nacional de Microbiología, Av. Vélez Sársfield 563, Buenos Aires, Argentina).*

1) First, the inhibition of the antidiphtheric toxin, purified by the action of the protamins, is studied. It is proved that in special conditions, this toxin loses 50 % of its primitive toxicity. If, before precipitating the toxin, zinc salt is added, the precipitation of the toxin becomes quantitative, and no toxins are found in the supernatant. This interesting fact, enables us to make trials with toxoids (antidiphtheric and tetanic) which as we shall see, give encouraging results.

2) Once the guinea-pigs are vaccinated with suspensions of protamin anatoxin, they gave the same immunizing results, as with anatoxin alone. The sera of the guinea-pigs in both batches, gave less than 1 a. u., for diphtheria, and 0.5 a. u. for tetanus. The protamin-anatoxin mixture or combination, does not prevent the rapid elution of the anatoxin, and does not, therefore, reinforce the immunizing power.

3) If a zinc salt is added, in the conditions specified in this paper, the anatoxins are precipitated with the protamin in a quantitative way. The diphtheric-tetanic combined vaccine obtained, meets with the specifications required by the "National Institute of Health". Half a human dose, i. e., 25 Lf of diphtheric toxoid, and 5 Lf of tetanic toxoid, give an immunologic response in guinea-pigs of 2 a. u. in diphtheria, and 2.5 a. u. in tetanus.

4) If, to the former vaccine, an inorganic bracket is added $Al(OH)_3$ in the same conditions already specified, and with the described technique, the serum of the guinea-pigs immunized in six weeks, 7 a. u. in diphtheria and 2.5 a. u. in tetanus is found. This inorganic bracket, gives an even greater value to the antidiphtheric immunization, and is maintained practically the same, as to tetanus.

APARECIO

ELEMENTOS DE FISIOLOGIA HUMANA

(The Living Body)

por

C. H. BEST y N. B. TAYLOR

Traducción de los doctores Samuel Middleton, Bjorn Holmgren y Daisy Benítez del Instituto de Fisiología de la Universidad de Chile.

La traducción al castellano de la 4ª edición inglesa de este importante texto satisface una imperiosa necesidad de la educación biológica en los países de habla española y será de gran provecho para la introducción al estudio de la fisiología. El libro consta de 744 páginas, 447 figuras y 16 láminas en colores. Incluye además un índice analítico de materias.

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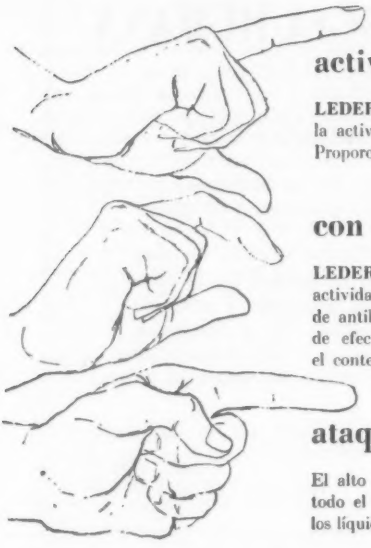

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milímetro	mm	mm	mililitro	ml	ml
micrón	μ	μ	kilogramo	kg	kg
milimicrón	$m\mu$	$m\mu$	gramo	g	gm
Angström	Å	Å	miligramo	mg	mg
microgramo	μg	μg	miliequivalente	mEq	mEq
hora	h	hr	Curie	c	c
minuto	m	Min	Milicurie	mc	mc
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